Tau as a biomarker of neurodegenerative diseases

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Abstract
Summary

The microtubule associated protein Tau is mainly expressed in neurons of the central nervous system and is crucial in axonal maintenance and axonal transport. The rationale for Tau as a biomarker of neurodegenerative diseases is that it is a major component of abnormal intraneuronal aggregates observed in numerous of these diseases named Tauopathies, including Alzheimer's disease. The molecular diversity of Tau is very useful when analysing it in the brain or in the peripheral fluids. Immunohistochemical and biochemical characterisation of Tau aggregates in the brain allows the post-mortem classification and differential diagnosis of Tauopathies. As peripheral biomarker of Alzheimer's disease in the cerebrospinal fluid, Tau proteins are now validated for diagnosis and predictive purposes. For the future, the detailed characterization of Tau in brain and in peripheral fluids will lead to novel promising biomarkers for differential diagnosis of dementia and monitoring of therapeutics.

Author Keywords Alzheimer's disease ; biomarker ; microtubule-associated Tau protein ; neurofibrillary degeneration ; phosphorylation ; tauopathies

Introduction

Tau protein is mainly expressed in the neurons of the central nervous system where it exerts a role in stabilizing microtubules, key components of axonal transport and in signal transduction. Tau alterations are observed in numerous neurodegenerative diseases at different levels: at the gene level with mutations responsible of rare familial dementia or polymorphisms associated with the increased risk of developing syndromes with parkinsonism; at the mRNA level, with altered alternative splicing leading to abnormal pattern of Tau isoforms in the brain; and at the protein level with in particular abnormal phosphorylation and cleavage leading to intraneuronal aggregation of Tau. These alterations are found among more than 20 different neurodegenerative disorders named Tauopathies, including Alzheimer's disease (AD) and non Alzheimer's type of dementia (progressive supranuclear palsy (PSP), frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), corticobasal degeneration (CBD), frontotemporal dementia (FTD) and Pick's disease (PiD) (for review see [1]). The diagnosis of these diseases remains difficult because of the heterogeneity and the partial overlap of their clinical presentations. With the aging of population, the incidence of some of these diseases, mainly AD is rapidly increasing and the need of biomarkers for a reliable diagnosis is tremendous. The molecular diversity of Tau, evolving from alternative splicing and from post-translational modifications, makes it well suited as a biomarker of these neurodegenerative disorders. The morphological and biochemical characterisation of Tau inclusions has lead to a huge improvement of the post-mortem differential diagnosis of these diseases. However, more recently, the development of sensitive assays for quantifying Tau in cerebrospinal fluid (CSF) also demonstrated its great interest as peripheral biomarker of neurodegenerative diseases, mainly AD. Tau inclusions in the brain associated with neuronal damages lead to the leakage of abnormal forms of Tau in the CSF resulting in quantitative and qualitative changes in CSF-Tau composition. As the brain lesions develop very early during the disease course even before the first clinical symptoms appear, CSF-Tau is not only a useful diagnostic marker in the advanced stages of the disease but also a prognostic marker in the earliest stages when clinical expression is weak. An illustration of the different applications of Tau protein analysis at different stages of AD disease is illustrated in Figure 1 and shows the broad possibilities presented by these biomarkers.

THE MICROTUBULE ASSOCIATED PROTEIN TAU

FROM THE GENE TO THE ISOFORMS

Tau is encoded by a single gene named MAPT , located on chromosome 17q21 (Figure 2A). MAPT gene belongs with several other genes to a chromosomic region flanked by low copy repeats (LCRs) that are susceptible to chromosomal rearrangements such as deletions, duplications or inversion [2]. From the 5'UTR to the end of the 3'UTR it spans 133.9 kb and contains 16 exons. There are more than 200 single nucleotide polymorphisms (SNPs) covering MAPT . More than 150 are in complete linkage disequilibrium (LD) with each other with an LD measure greater than 0,8 (http://www.hapmap.org) and define an extended haplotype that cover the entire MAPT gene (Figure 2B) [3]. Only twenty of them show a LD measure smaller than 0,4. This haplotype even spans to a region covering ~1,8Mb [4]. The H2 haplotype is much more rare than the H1 haplotype in healthy individuals, showing different prevalence among ethnic groups and results

from H1 by the inversion of a ~900 kb segment resulting from a rearrangement between LCRs [2]. In the central nervous system (CNS), two transcripts of 2 kb and 6 kb arise from utilization of two alternative polyadenylation sites, the 2 kb mRNA targets Tau to the nucleus and the 6 kb encodes the major form in axons [5 , 6]. Alternative splicing is tissue specific and developmentally regulated : in the CNS, exons 2, 3 and 10 are alternatively spliced (Figure 2C) leading to one fetal isoform, which is still expressed in adult stage, and five additional adult isoforms. The six Tau isoforms are made of 352-441 amino acids with molecular weight of ~37-46 kDa (Figure 2D). Brain Tau proteins can be subdivided in four regions: an amino-terminal region named projection domain which is acidic, a proline-rich region followed by imperfect microtubule binding repeat motifs (encoded by exons 9 – 12) and a short carboxy-terminal region. Each isoform is characterized by the the length of the N-terminal domain and by the presence of three or four repeat motifs, depending respectively on exon 2/3 or exon 10 alternative splicing. The diversity of Tau isoforms is further increased by various posttranslational modifications : phosphorylation (developed further), O-glycosylation, ubiquitination, nitration, glycation (for review, see [7]).

TAU FUNCTION IN THE CNS

One major biological function of Tau is to build an ordered microtubule network in axons, which is essential for the axonal transport. The large carboxy-terminal microtubule binding domain promotes microtubule assembly and maintains the stability of the previously formed microtubules through repetitive sequences. The amino-terminal region together with the proline-rich domain project from the microtubules surface to adjacent microtubules and is proposed to determine spacing between microtubules. Tau proteins would therefore contribute to the parallel ordered organization of microtubules in axons.

More recently, other important roles of Tau are suggested by the interactions of the N-terminal domain with protein partners. Interactions with motor proteins such as kinesin-1 [8] and dynactin/dynein complex [9] suggest a role in the dynamic of axonal transport. The binding to SH3 containing proteins such as Fyn, a Src family kinase [10], phospholipase C-gamma 1 or p85-apha subunit of PI-3K [10] supports a role of Tau in neuronal signal transduction. Association of Tau amino-terminal region with several of its interacting partners is regulated by phosphorylation and is further discussed in the phosphorylation section. Finally, Tau proteins interact with the plasma membrane or with cytoskeleton proteins such as actin, spectrin and neurofilament proteins suggesting a role in neuronal cell architecture.

The polypeptide sequences encoded by alternative spliced exons modulate specific Tau functions: the amino-terminal inserts encoded by exons 2 and 3 affect the microtubule spacing and the fourth microtubule domain encoded by exon 10 modulates interactions to microtubules. Tau isoforms containing 4 repeats (4R-Tau) bind to microtubules with a greater affinity (for review see [1] and are more efficient at promoting microtubule assembly than isoforms containing 3 repeats (3R-Tau). This suggests that Tau isoforms have specific functions [11]. The ratio of isoforms is probably important for correct cell function and is not the same in all neurons. For example, Tau mRNAs containing exon 10 are not found in granular cells of the dentate gyrus [12].

TAU PHOSPHORYLATION

Eighty-five potential phosphorylation sites are distributed along the longest Tau isoform and are essentially located in regions surrounding the repeat-binding motifs. According to the latest extensive analysis of Tau phosphorylation [13] and that of one previously published review [14], 71 among the 85 putative phosphorylation sites that can be phosphorylated in physiological or pathological conditions. A total of more than 20 protein kinases can phosphorylate Tau proteins : an extensive review of these kinases is found in [15]).

For some of these kinases, the effect on Tau function has been described. The proline directed protein kinases (PDPK) like the cyclin-dependant kinases 1 and 5, MAPK and GSK families are involved in modulation of Tau binding to microtubules (Thr231 and Ser396) and also in signal transduction (for review, [1]). The non-PDPK includes a number of kinases also involved in signal transduction where Tau may act as a linker or modulator. However, among inserm-00375314, version 1 - 14 Apr 2009 14/04/200914:04:54 7 them, MARK kinases are strictly involved in the regulation of Tau binding to microtubules by phosphorylating specific motifs (KXGS) within the microtubule-binding domains [16]. Noteworthy, GSK3β phosphorylates MARK and inhibits MARK activity, making a functional link between the two groups of kinases and showing that GSK3β and MARK both contribute to regulated the binding of Tau to microtubule. Regarding the tyrosine protein kinases, studies have determined that human Tau Tyr18 and Tyr29 are phosphorylated by the Src family tyrosine kinase Fyn [17 , 18]. More importantly, GSK3β is shown to phosphorylate Tau and reduce the binding of Fyn, PLC-γ 1, p85α subunit of PI3K [10]. Finally, cotransfection of Tau and tyrosine protein kinases showed that even if Tyr-18 is the major site for Fyn phosphorylation, Tyr-394 is the main residue for Abl. [19].

TAU ALTERATIONS IN NEUROLOGICAL DISEASES

MAPT DELETION AND DUPLICATION

Both duplication and deletion of the 17q21.31 region containing the MAPT gene have been reported. De novo microdeletions have been identified in several patients with developmental delay, hypotonia and learning disability [20 -23], the prevalence of this new syndrom has been estimated to 1/13000 and 1/20000 [20]. One microduplication reciprocal to the microdeletion was described in a case of severe psychomotor developmentally delay with dysmorphic features [24]. This suggests that dosage sensitive genes are present in this

chromosomal region, perhaps the MAPT gene. Further studies will be needed to evaluate the potential detrimental effects of Tau haplo-insufficiency or triplication.

MAPT GENE MUTATIONS

In AD, no MAPT mutation has been described. However, study of familial cases of non Alzheimer's Tauopathies has lead to the discovery since 1994 of at least 43 MAPT mutations that cause very rare autosomal dominant inherited dementia named FTDP-17 (Frontotemporal dementia with parkinsonism linked to chromosome 17) (Tau mutations are listed on www.molgen.ua.ac.be/FTDmutations) [25]. Approximately one hundred families with MAPT mutations have been reported in the world. The clinical features of these dementia are variable but are usually characterized initially by behavioural and motor disturbances that are later associated with cognitive impairment. The most common mutations, accounting for approximately 60% of known cases, are two missense mutations P301L, N279K and one splice site mutation (exon10 +16) (Figure 2B)[25]. MAPT missense mutations have also been identified in PSP, CBD and Pick's disease. The vast majority of these mutations are located in the coding region or close to the splice-donor site of the exon 10. The primary effect of most mutations appears to be a reduced ability of Tau to interact with microtubules, but alternatively some of them can affect exon-10 splicing leading to an imbalance in the expression of the different Tau protein isoforms. Furthermore, increased Tau self-assembly has also been detected in most of these mutated Tau proteins. In almost all these familial cases, accumulation of hyperphosphorylated Tau protein in neurons has been observed (for review, see [26]).

MAPT GENE POLYMORPHISMS

The first evidence that the H1 haplotype is associated to an increased risk for several Tauopathies was shown for PSP (progressive suplanuclear palsy). Further studies were then investigated in other Tauopathies like AD, FTD, CBD or Pick's disease (for review, see [3 , 27]) : except for CBD where a significant overrepresentation of H1 haplotype in affected patients was observed, results among studies were controversial and do not allow to conclude inserm-00375314, version 1 - 14 Apr 2009 14/04/200914:04:54 9 that Tau gene polymorphism is a risk factor for theses diseases. In Parkinson's disease, two recent studies showed that the H1 haplotype was associated with a significantly increased risk for Parkinson's disease (PD) [28 , 52]. Moreover, the sub-haplotype H1c, located in the promoter region of Tau gene, has been shown to be a more specific risk factor for PSP [29]. Association analysis of this H1c subhaplotype with AD [30 , 31] is controversial. The exact pathogenic mechanism by which MAPT polymorphisms can cause an increased risk is unknown but one hypothesis is that they can influence Tau expression and splicing. The H1 haplotype, and particularly the H1c, showed significantly greater expression in vitro of Tau proteins than H2 [32] but this was not confirmed in vivo on unaffected post-mortem human brain [33]. However, the H1 haplotype, appears to express up to 43% more exon 10+ transcripts than H2 [34 -36]. Interaction between MAPT gene and other potential pathogenic genes was also reported. For instance, the combination of risk genotypes of both MAPT and αclein has been recently shown to approximately double the risk for the development of PD [37].

ALTERED MAPT SPLICING

Dysregulation of splicing can be caused either by alteration of cis-sequences on MAPT gene or alteration of MAPT splicing factors. The importance of MAPT splicing in the physiopathology of Tauopathies was first highlighted by FTDP-17 mutations, located within or in the vicinity of exon 10, affecting Exon 10 Tau splicing and placing it upstream of the dementia process. Regarding alteration of MAPT splicing factors, the disease model is myotonic dystrophy.

Myotonic dystrophy (DM) is the most prevalent form of adult-onset muscular dystrophy. Beside the cardinal muscle symptoms (myotony, progressive myopathy), other common troubles affect multiple organ systems (heart, genital tract, eyes, endocrine system), including the central nervous system : cognitive impairment, including memory, visuo-spatial recall, verbal scale, with cortical atrophy of the frontal and the temporal lobe and white matter lesions are often described in both DM1 and DM2 [38 , 39]. DMs are inherited autosomal dominant disorders caused by dynamically instable CTG or CCTG expansions in the 3'UTR of DPMK or the first intron of ZNF9 in DM1 and DM2, respectively [40]. A disruption of MAPT splicing in DM1 and DM2 is observed together with a pathological aggregation of Tau proteins (further developed in the paragraph "Tau as biomarkers of brain pathology") [41 -45]. In several cortical brain areas of a DM1 patient, exon 2 splicing is altered (Figure 3A) with a reduced inclusion of exon 2 and a relative increase of 2-3- transcripts (Figure 3B). Missplicing of exon 10 in DM1 is inconstantly observed [43 , 44]. Splicing alteration in DM1 and DM2 are due to a loss of function of a MAPT splice factor, MBNL1, caused by the DM1 or DM2 mutation [46] implicating a RNA-mediated trans mechanism (for review, see [40 , 47]).

Several studies suggest that altering MAPT splicing could be responsible of central nervous dysfunction by altering the Tau isoform ratio: overexpression of Tau 4R in neuroblastoma cells showed enhanced susceptibility to cell death [48] and animal models with excess of exon 2/3 inclusion causes gliopathy and spinal cord degeneration [49]. Thus, abnormal Tau splicing is sufficient to cause neurodegeneration, although the exact mechanism that leads to clinical symptoms is not clear. A disruption of proper balance of Tau

isoforms is also observed in several sporadic Tauopathies, including FTD, PSP and CBD [50], PiD [51], PD [52] and is inconstantly observed in AD [53 -56]. This suggests that a common toxic mechanism is shared in all these Tauopathies and that restoring the isoform balance may be a novel therapeutic strategy [6].

TAU AGGREGATION

In more than 20 neurodegenerative disorders, referred to as Tauopathies, Tau proteins aggregate in the affected cortical and subcortical brain regions [57], forming intraneuronal inclusions. The most known is AD and other Tauopathies include PSP, FTDP-17, CBD, FTD and PiD. Biochemically, the inclusions contain aggregated Tau proteins characterized by abnormal phosphorylation and/or abnormal splicing. All the isoforms are capable of polymerization into fibrillar structures such as those present in AD. During long time, it was admitted that Tau aggregation correlates with neuron loss and neuron toxicity; this was suggested by the fact that Tau aggregate spreading in AD brain increases with cognitive decline. More recently, some studies suggested that Tau aggregation can be dissociated from neuronal loss [58 , 59] and can be even be protective [60] as suggested for Huntingtin in Huntington disease [61]. Small Tau oligomers could be involved in neurodegeneration [62].

ALTERED TAU CONFORMATION

Specific conformational changes of Tau are observed at early stages of AD. In FTDP-17, Tau proteins are altered. The conformational changes may render Tau more susceptible to phosphorylation. Abnormal conformation of Tau has been reported to be toxic in cell models and in transgenic mouse models [63]. When the aggregation occurs, fibrils take a very well structured organization corresponding to paired helical filaments (PHF) in neurofibrillary tangles for AD.

TAU HYPERPHOSPHORYLATION

The most prominent modification of Tau in all Tauopathies is abnormal phosphorylation characterized by i) high level of phosphorylation on epitopes localized at the half-N-terminus and C-terminus outside the microtubule binding domains (e.g. epitopes pTau181, pTau199, pTau231 pTau396 and pTau404) and ii) additional pathological phosphorylation sites (e.g epitope pTau422) (phosphorylation sites on PHF-Tau are reviewed in [14]).

One consequence is that the most striking difference in between post-mortem Tau and PHF-Tau proteins is their electrophoretic profiles. Normal “post-mortem” Tau proteins are resolved as 6 main bands (45-67 kDa) whereas more acidic hyperphosphorylated PHF-Tau are resolved as four bands comprised between 60 and 74 kDa (see further, Figure 4). In normal brains, the pTau epitopes are rapidly dephosphorylated during the post-mortem delay: this effect is likely due to the rapid drop of ATP and activation of phosphatases. Conversely, in AD brains, this dephosphorylation does not occur. It may be due to 1) the aggregation of Tau proteins into filaments that render them inaccessible to phosphatases; 2) phosphatases that are no more activated in degenerating neurons or 3) phosphatases activity is already decreased [64]. Nevertheless, hyperphosphorylation and abnormal phosphorylation may useful as diagnostic markers. The most studied phospho-epitopes in CSF are pTau181 and pTau231. It is well established that pTau231 appears early in AD, occurring before the formation of PHF in the neurons of hippocampus. Phosphorylation at threonine 181 and serine 199 occurs later, and theses are only found to any appreciable extent in intracellular tangles [65]. Reactivity to TG3, an antibody that recognizes phosphothreonine 231, is found in pretangles, intracellular tangles and extracellular tangles and so is present at all stages of the disease. The additional pathological phosphorylation sites can be detected using antibodies that recognize PHF-Tau and not normal or “native” Tau, such as AD2 that recognizes the phosphorylated Serine 396 and Serine 404 or AT100 that recognizes the phosphorylated Threonines 212-217 and Serine 214. Nonetheless, to generate the pathological phospho-sites at AT100 epitope a sequential phosphorylation by two kinases [66] is necessary suggesting that several kinases may be deregulated in AD. Among the kinases described above, GSK3-β and cdk5 play an important role in regulating Tau phosphorylation under pathological conditions.

Hyperphosphorylated Tau-epitopes are not the same in all Tauopathies: in PiD, Pick bodies, the neuropathological feature of this disease and composed of aggregated Tau proteins, are not detected by the monoclonal antibody 12E8 raised against the phosphorylated residue Ser262/Ser356 whereas in other neurodegenerative disorders, this phosphorylation site is detected [67 , 68]. The lack of phosphorylation at Ser262 and 356 sites is likely to be related to either a kinase inhibition in neurons that degenerate in PiD or an absence of these kinases within degenerating neurons.

Hyperphosphorylation is associated with a loss of microtubule binding capacity and a consequent accumulation in neuronal bodies. Tau hyperphosphorylation correlates with neurodegeneration in the brain of transgenic mice overexpressing an FTDP-17 mutant [69]. Tau hyperphosphorylation is a relatively early event in the development of AD [70]. Phosphorylated Tau appears to be more resistant to proteolysis by different proteases [71] and therefore could accumulate in neurons and contribute to Tau toxicity.

TAU CATABOLISM

Tau is cleaved in vivo by at least two groups of proteases, caspases [72 , 73] and calpains [74] and is degraded by the proteasome [75] . Tau fragments generated by different proteases may differ in their ability to assume pathological conformations, to aggregate and to induce neurotoxicity [76 , 77]. It has been shown that successive cleavage events occur on Tau protein during the course of NFT evolution in AD [76]. In AD and in other neurodegenerative disorders, Tau truncation by caspases is an early event [78]. Calpains also become over-activated because of elevation of cytosolic $Ca2+$ levels. Inhibition of the proteasome leads surprisingly to an increased Tau degradation [79]. Once proteolysed, Tau fragments turn into effectors of apoptosis and initiate and accelerate DNF development [80 , 81]. The number of aggregates positive for caspase 6 truncated Tau fragments has been inversely correlated to cognitive score during aging and early stages of AD [78]. More recently, a 35 kDa C-terminal Tau fragment, lacking the N-terminus of Tau but containing four microtubule-binding repeats was shown to be present only in neurodegenerative disorders in which 4R was overrepresented: it was detectable in PSP, CBD and 4R forms of FTDP-17 but was absent of controls, AD and PD brains [82]. All these observations suggest that Tau fragments are potential biomarkers of AD.

TAU PROTEINS AS BIOMARKERS OF BRAIN PATHOLOGY

Tau aggregates form intraneuronal inclusions of typical morphologic features specific of the Tauopathies: neurofibrillary tangles, neuropil threads, dystrophic neurites of neuritic plaques and Pick bodies. The generation of specific antibodies using antibodies that recognize PHF-Tau and not normal or “native” Tau, such as AT100 and against amino acid sequences corresponding to alternative spliced exons 2, 3 and 10 allowed the characterization of Tau aggregates in Tauopathies, using immunoblotting and immunohistochemistry. Tau aggregates differ in both Tau phosphorylation and isoform content, which enables a molecular classification of Tauopathies. We propose a classification composed of four classes of Tauopathies, defined depending on the type of typical Tau electrophoretics profiles of aggregates, constituting disease specific biochemical signatures or “Bar Code” for neurodegenerative disorders [15 , 83].

Class I: All Brain Tau isoforms are aggregated

Class I is characterized by a pathological Tau quartet at 60, 64 and 69 kDa and a minor pathological Tau at 72/74 kDa [84]. This pathological Tau quartet corresponds to the aggregation of the six Tau isoforms [85 , 86]. This classification is summarized on Figure 4 . Adjacent to the Western blot image are the isoform composing each bands (For review see: [1]). Using histochemistry, aggregates of class I Tauopathies positively react with AD2, and antibodies directed against exon2 and exon 10 (Figure 4A).

Class II: Tau isoforms containing the exon 10 encoding sequence aggregate

The class II profile is characterized essentially by the aggregation of Tau with four microtubule-binding domains (Figure 4B). This pathological Tau profile is observed in corticobasal degeneration (CBD), Argyrophilic grain dementia (AGD), progressive supranuclear palsy (PSP), and Frontotemporal dementia linked to chromosome 17 due to Tau gene mutations [87 , 88]. PSP, CBD and AGD are rare atypical Parkinsonism disorders which classification was recently updated by the Consortium for Frontotemporal lobar degeneration [89].

Class III: Tau isoforms lacking the exon 10 encoding sequence aggregate

Class III of Tauopathies includes PiD and autosomal dominant inherited FDTP-17 (Figure 4C). Pick’s disease is a rare form of neurodegenerative disorder characterized by a progressive dementing process. Early in the clinical course, patients show signs of frontal disinhibition [90]. Neuropathologically, Pick’s disease is characterized by the presence of typical spheroid inclusions in the soma of neurons called Pick bodies. Pick bodies are labeled by Tau antibodies, with a higher density in the hippocampus than in the frontal and temporal cortices. The pathological Tau profile of PiD contrasts with that of class II Tauopathies, with the pathological Tau isoforms consisting essentially of the 3R Tau isoforms. Immunohistologic staining of those aggregates is postive with AD2 and anti-exon2 antibodies and negative with anti-exon10 antibody.

Class IV: Tau isoform lacking exon 2, 3 and 10 principally aggregate

Class IV is represented by myotonic dystrophy of type I and type II (Figure 4D). Neuropathological lesions, such as neurofibrillary tangles, have been observed in adult DM1 individuals over 50 years of age. The pathological Tau profile of DM1 is characterized by a strong pathological Tau band at 60 kDa and to a lesser extent, a pathological Tau component at 64 and 69kDa. This typical pathological Tau profile is reflected by a reduced number of Tau isoform expression in the brain of individuals with DM1, at both the protein and mRNA levels [44]. The analysis of multiple brain regions of one genetically confirmed DM2 patient aged of 71 years, showed some neurofibrillary degenerating processes. Using specific immunological probes against amino acid sequences corresponding to exon 2 and exon 3 corresponding, the neurofibrillary lesions were shown to be devoid of Tau isoforms with Nterminal inserts (Figure 4D) [45]. An altered splicing of Tau with a reduced expression of Tau isoforms containing the N-terminal inserts characterizes both DM1 and DM2.

Overall, it demonstrates that the central nervous system is affected and that DMs are real Tauopathies. The direct relationship between the altered splicing of Tau and neurofibrillary degeneration in DM remains to be established. Indeed, such an altered splicing of Tau is commonly observed in FTDP-17 and considered as reminiscent to neurofibrillary degeneration and Tauopathies.

TAU PROTEINS AS PERIPHERAL BIOMARKERS OF NEURODEGENERATIVE DISEASES

The generation and characterization of highly sensitive and specific antibodies to the microtubule-associated protein Tau has not only lead to a better understanding and detailed characterization of Tau in normal and diseased brain, but has also advanced considerably our understanding of Tau as biomarker in circulating fluids for a number of neurological conditions. While the first observations on the presence of Tau in CSF were based on several sandwich immuno-assays, improvement in highly sensitive western-blotting techniques and the use of immuno-capture mass-spectrometric techniques has lead to the unequivocal characterization of Tau in CSF.

CSF TOTAL TAU : A BIOMARKER OF NEURONAL DEGENERATION

The presence of Tau in cerebrospinal fluid (CSF) was first described in 1993. Quantitative analysis of total Tau in CSF (CSF-tTau) was developed, using ELISA assays with different Tau antibodies that detect all Tau isoforms independently of their phosphorylation state (see Figure 5 for antibodies used in these assays). These ELISAs showed that CSF-tTau displays an age-associated increase in non-demented control individuals [91] and therefore age-adjusted reference values should be used when CSF-tTau is used diagnostically. All the data of more than 50 studies including over 2500 AD patients and 1300 controls have consistently demonstrated increased CSF-tTau levels in AD, with mean levels 2-3 times higher compared to healthy controls (for review, see [92]). The most commonly used is a commercial sandwich ELISA kit (AT270-HT7), that shows a good specificity (90%) and sensitivity (81%) discriminating AD from age-matched controls [93]. Age-adjusted reference values have been established to improve discrimination between AD and controls: <300 pg/ml in the group 21- 50 years of age, <450 pg/ml in the group 51-70 years of age and <500 pg/ml in the group 71- 93 years of age [94]. There is evidence that CSF-tTau reflect the presence of the neuropathological hallmarks of AD from neuropathological studies [95].

The increase of CSF-tTau comes probably from leakage of Tau from damaged neurons into CSF and therefore reflects the intensity of neuronal damage and degeneration. This is assumed from the fact that in acute conditions as stroke or Creutzfeld Jacob disease (CJD), there is a marked increase in CSF-tTau. After acute ischemic stroke, CSF-tTau level increases transiently (Table 1). The highest CSF-tTau levels with values of 10-50 times higher than in controls, were revealed in CJD, a disorder with very intense neuronal loss. Using a cut-off value of 1300 pg/ml, several studies on differentiation of CJD from AD and other dementia showed that CSF-tTau level is a highly discriminative marker, which has recently been confirmed in a neuropathological study to be as good as the established 14-3-3 marker for CJD [96]. Elevated values for CSF-tTau have also been found in bovine variant of CJD [97]. Increase of CSF-tTau levels are observed in several other acute neurological conditions, like severe malaria [98], Wernicke's encephalopathy [99], pediatric patients with brain tumor, hydrocephalus or serious CNS infections [100].

Studies assessing CSF-tTau in non-AD dementia such as FTD, PSP, CBD, Lewy Body Dementia (LBD), PDD and Amyotrophic Lateral Sclerosis (ALS) give contradictory results. In FTD cases, CSF-tTau has been found increased by some investigators and reported normal or reduced by others [101 -103]. In LBD, CSF-tTau has been found to be increased in some studies [104] and not in others [105 , 106] including one on an autopsy verified series [107]. Increased CSF-tTau has been also reported for PDD patient [108 , 109]. In PSP and CBD syndrome, CSF-tTau levels were found within normal range [109] or increased [92]. In ALS, inconstant increased CSF-tTau levels are described [110 , 111]. This suggests that presence of Tau pathology does not necessarily result in higher CSF-tTau levels. In vascular dementia (VaD), some studies have reported normal CSF-tTau levels whereas others have found an elevation [92]. These conflicting results might be caused by selection bias as high concomitant AD pathology was observed at autopsy in patients clinically diagnosed as VaD [112].

Normal CSF-tTau are found in several neurological diseases such as other type of dementia (alcoholic dementia), chronic neurological disorders (parkinson's disease [without dementia], multiple sclerosis) and psychiatric disorders (depression) [113 , 114].

In conclusion, CSF-tTau has a clear diagnostic value over that of clinical criteria of AD for the discrimination of AD from normal aging, depression, chronic neurological disorders, and alcoholic dementia and possibly VaD. However for differential diagnosis from other non-AD dementias (FTD, CBD and LBD), the diagnostic value of CSF-tTau is insufficient.

CSF-pTAU: A BIOMARKER OF HYPERPHOSPHORYLATION

The use of phospho-dependent antibodies showed that phosphorylated Tau is recovered in the CSF and that several phospho-epitopes are present (for review see [92], such as Thr181, Thr235, Ser396 and Ser404. A moderate to marked increase in CSF-pTau compared to controls has been found for all the different ELISA methods based on these antibodies (for review, see [92], [93]). Pooling 13 different

papers including more than 1000 AD patients and 500 controls, the mean sensitivity to discriminate AD from controls was 81% and the specificity 91% [115]. A study compared the diagnostic performances of pTau181, pTau199 and pTau231 in the same samples [116]: all three pTau assays discriminated as well AD from normal aging.

There is no change in CSF-pTau after acute stroke [92], although there is a marked increase in total Tau. Furthermore, CSF-pTau levels are normal or only mildly increased in CJD despite a huge increase in total Tau [117]. This suggests that pTau is probably not simply a marker of neuronal loss but reflects the phosphorylation state of Tau. Normal CSF levels of pTau are found in chronic neurological disorders (parkinson's disease, multiple sclerosis or ALS) and psychiatric disorders (depression) [91 , 118] and also in most cases of other dementia such as VaD, FTD and LBD [92 , 116]. This implies that the specificity of pTau to differentiate from other dementias is higher than for total Tau, reaching more than 80% [119]. However, differences were observed between phospho-epitopes in their potential to discriminate AD from other dementias: the best discrimination between AD and FTD being obtained with pTau231, between AD and DLB with pTau181 [119 , 120]. The use of phospho-Tau as a marker to distinguish dementia with Lewy bodies from fronto-temporal lobe dementia [120] also shows promising results, which needs to be further confirmed and extended [102 , 121]. Finally, it is interesting to note that Tau pathology and hyperphosphorylation does not necessarily result in increased CSF-pTau levels.

INFLUENCE OF GENE POLYMORPHISMS ON TOTAL TAU AND pTAU LEVELS

Interindividual variations of CSF Tau and pTau levels are important in controls and in AD patients. Therefore, some investigators studied the impact of polymorphisms known to be associated to an increased risk of developing AD on these levels. Neither apoEεe nor MAPT H1/H2 haplotype influenced CSF of total Tau or phospho-Tau [122 -126]. Concerning association of MAPT H1c haplotype with CSF-tTau levels, results are contradictory [126 , 127]. One study showed that two very rare H1 haplotypes were correlated with higher CSF-tTau levels. However, a combined genotype and CSF-tTau or CSF-pTau may be useful for the diagnosis or prognosis of other neurodegenerative disease. Finally, a very interesting recent observation was that a polymorphism of the kinesin light chain 1 gene (KNS2), that has been demonstrated to be associated to an increased risk of developing AD [128], was associated with high CSF pTau levels in MCI patients who converted to AD [129].

pTAU : A PREDICTIVE BIOMARKER OF AD

The rationale for CSF-tTau and CSF-pTau as predictive biomarkers of AD is that pTau is a major component of pre-tangle PHF and mature neurofibrillary tangles that are present within the brain even before the onset of AD [70]. During this preclinical period there is a gradual loss of axons and neurons, and at a certain threshold the first symptoms, most often impaired episodic memory, appear. At this stage patients do not fulfil the criteria for dementia and may be diagnosed with mild cognitive impairment (MCI). Patients with MCI can evolve to AD or remain with their MCI. The former are named converters or prodromal Alzheimer patients [130].

Several studies showed that high CSF-tTau and CSF-pTau levels were found already in early AD and the performance of these biomarkers in AD cases with MMSE (Mini-Mental State Examination) scores above 23-25 have been found to be similar to those in more advanced AD [131]. Moreover, in MCI cases that developed AD during clinical follow-up, these biomarkers are already altered with sensitivities similar to those found in AD cases with clinical dementia [132]. Longitudinal studies over periods ranging between 2 and 6 years in subjects with MCI showed that pTau levels were significantly increased only in the MCI converters and that pTau at different epitopes including Thr181, Thr231 and Ser 199 is the most reliable predictor of the decline from MCI to AD, with a sensitivity for prediction of 66- 100% and a specificity of 66-78% [133 -139]. It is important to note that as only ~15% of MCI cases progress to AD each year [140], only very extensive follow up (more than 5 years) are needed to ascertain which patients will not develop dementia. Until today, most studies were done on shorter follow-up periods; it is probable that the specificity data are too low. Finally, these data on sporadic AD are confirmed by studies of familiar AD showing that increased CSF-tTau and CSF-pTau levels are elevated in presymptomatic PSN-1 or APP mutation carriers [141]. The very early diagnosis of AD at a presymptomatic stage of the disease will be the basis for initiating preventive treatments in the future.

CLINICAL SIGNIFICANCE OF CSF TAU AND pTAU LEVELS IN NEURODEGENERATIVE DISEASES

Dementia disorders are characterized by progressive cognitive and functional decline. While the increase in CSF levels of Tau in patients with AD was rapidly confirmed, its use as a diagnostic marker is based on the sustained unchanged levels over longer periods of time during the disease process (see Table 1 for an overview of published data). In most studies, no significant changes in CSF-tTau and CSF-pTau have been reported in follow-up studies in mid- and late-stage AD with CVs ranging from 6.1% in a 6 month follow-up study to 21% in a 1 year follow-up. Studies in other neurodegenerative diseases such as brain trauma, neurotoxicity, alcohol dementia the increase in CSF-tTau levels is transient (Table 1). While some of these studies in neurodegenerative diseases associated with neuronal/axonal loss seem controversial, timing of CSF sampling with respect to the transient change is obviously crucial to the interpretation of the results. Indeed longitudinal follow-up studies in patients with multiple sclerosis [142], Guillain-Barré syndrome [143] or traumatic brain injury [144] suggest that degree of elevated Tau is related to poor outcome and thus may have prognostic value. Thus in neurodegenerative diseases in which axonal damage is related to clinical outcome quantifying CSF-tTau might have prognostic value, while in AD, MCI-AD

and Creutzfeld-Jacob disease where the elevation of Tau is high and relatively stable, the increase of Tau levels has diagnostic and progonstic value [96 , 130 , 145].

INCREASED POWER OF CSF TAU BIOMARKERS WHEN COMBINED TOGETHER OR WITH OTHER BIOMARKERS

The combination of several CSF markers will increase the specificity of tests for discriminating dementia. Especially, the association of CSF amyloid peptide Aβthat reflect the presence of amyloid plaques in AD brains increases the diagnostic power of Tau proteins (for review, see [92 , 146 -148]).

Stable increased tTau/pTau levels do not only have value in differential dementia diagnosis [149], but might also be useful in early diagnosis. Also in this early stage of the disease the levels of CSF-tTau/pTau is stable over time (Table 1 [135 , 139 , 145 , 150]). To improve the reliability of early diagnosis of AD a combination of biomarkers will be required.

The association of 14-3-3 protein with CSF-tTau increases test specificity for the diagnosis of CJD [151]. Moreover, the ratio of pTau to total CSF-tTau was found to discriminate CJD from other neurodegenerative disease without any overlap [117]. Finaly, pTau and/or pTau/tTau ratio might be important markers to differentiate subtypes of CJD [97 , 152].

CSF-tTAU AND pTAU AS THERAPEUTIC MONITORING BIOMARKERS

Stable levels over longer periods of time are not only useful in early diagnosis but offers also potential as a surrogate biomarker. The definition of a surrogate endpoint is that a biomarker is intended to substitute for a clinical endpoint. It is expected that such an endpoint predicts clinical benefit based on epidemiologic, therapeutic, pathophysiological, or other scientific evidence [153]. Several small drug studies have already studied CSF-tTau/pTau in relation to drug treatment in AD patients and a number of these studies suggest that Tau/pTau levels can be ‘normalized/decreased' upon treatment (Table 2). In particular the reduction of CSF-tTau in immunotherapy might be particular relevant. Furthermore, association studies with single nucleotide polymorphisms suggest that the use of Tau measurements can further be improved taking into account those associations (Table 2). Also discoveries in other drug treatments such as the association of Tau mRNA levels with sensitivity to Paclitaxel treatment of breast cancer [154], might also be of relevance for Tau-related disorders [155] (Table 2). However, clearly this field of biomarker research is in its early phases and longitudinal studies are required to definitely demonstrate the relationship between change in Tau/pTau levels and clinical outcome.

CSF TAU FRAGMENTS: PROMISING DIFFERENTIAL BIOMARKERS OF DEMENTIA

Tau in CSF consists of partially proteolytic fragments ranging from 14 kDa to 55 kDa and full-size Tau. Based on identification of specific peptides it is clear that all isoforms found in the brain are present in the CSF. Tau fragments are mainly composed of two major polypeptides of 55kDa and 33kDa [109]. The 55kDa band contains a large domain of Tau extending from the N-terminal region (amino acid 159) to the carboxy-terminal region (amino acid 432). In contrast, the 33kDa band shares a similar amino-terminal epitope with that of the 55kDa band, but is truncated at the C-terminus of Tau. Those Tau fragments were shown to be present in the brain tissue [82]. A 14kDa fragment of Tau was also evidenced by Zemlan and collaborators using C-terminal Tau antibodies [156]. More recently, a characterization by mass spectrometry of Tau in human CSF identified 19 tryptic fragments, of which 16 are common to all isoforms and 3 specific of unique Tau isoforms [157]. The sequence of the largest form of human brain Tau is shown in figure 5 and summarizes the characterization of Tau in CSF.

In CSF of PSP patients, Tau ratio 33 kDa/55 kDa was significantly decreased when compared to controls and AD, FTD and CBD. This is consistent with the recent study in PSP brain identifying a Tau fragment specific of 4R isoforms [82]. The multiplicity of Tau fragments in CSF represents a great potential for differential diagnosis of dementia and therefore their analysis should be developed further in independent studies and on larger patient series. Thus, a detailed characterization of Tau in CSF of neurodegenerative diseases [157 , 158] may lead to simple biochemical tests that may assist in sometimes-difficult neurological diagnosis.

FUTURE PERSPECTIVE

For the use of Tau as biomarker in large clinical trials or in clinical practice, one important goal in the future years will be to develop sensitive methods to detect the very low inserm-00375314, version 1 - 14 Apr 2009 14/04/200914:04:54 25 concentration of Tau in the blood (~30 pMol) and even in urine. One promising techniques will be immunoPCR. High-confidence protein identifications in the HUPO plasma proteome have identified Tau in two paired plasma/serum samples, suggesting the presence of Tau in some of the samples [159]. Indeed a number of early reports using the tTau ELISA (AT270-HT7) assay or an assay for truncated Tau have reported increased Tau-like immunoreactivity in serum/plasma [160 -164] of patients with severe neurodegeneration. However, not all studies have reported increased Tau levels in serum [165], suggesting that sample pre-treatment and handling will be crucial in developing a reliable Tau assay in blood/plasma. The stable increased levels of CSF-Tau/pTau might be a unique feature of AD that will be reflected in the plasma of patients. A multiparametric approach will probably be needed to solve the problem of differential diagnosis of neurodegenerative diseases. Therefore, the development of new technologies like xMAP (Luminex®) allowing the simultaneous analysis of as much as hundred

biomarkers is promising. The diagnosis of neurodegenerative diseases will very probably rely on the definition of disease specific protein profiles.

EXECUTIVE SUMMARY

The microtubule associated protein Tau

• alternative splicing and posttranslational modifications leads to multiple Tau isoforms

• Tau proteins have major functions in the CNS in axonal transport and possibly in signal transduction

• Tau phosphorylation modulates its activity in the CNS

Tau alterations in neurodegenerative diseases

• MAPT mutations are responsible of rare forms of dementia

• MAPT polymorphims are associated with some dementia like PSP, CBD, PD or AD

• Altered MAPT splicing is observed in genetic forms of dementia and in sporadic Tauopathies. The consequently imbalance of Tau isoforms could be responsible of neuron dysfunction.

• Tau aggregation is a major feature of a class of dementia named Tauopathies.

• Altered Tau conformation is observed in Tauopathies

• Tau abnormal hyperphosphorylation is a main stigmat observed in Tauopathies.

• Loss of Tau protein expression is observed in some types of FTDs.

• Tau protein cleavage is observed in the brain of patients with Tauopathies.

Tau proteins as biomarkers of brain pathology

Immunohistology of Tau inclusions and electrophoretic analysis of Tau aggregates allows a classification of Tauopathies usefull for post-mortem differential diagnosis.

Tau proteins as peripheral biomarkers of neurodegenerative diseases

• CSF total Tau levels has a clear diagnostic value for AD but is insufficient for differential dementia diagnosis

• CSF phospho-Tau levels have an improved diagnostic value for AD compared to total Tau levels and a potential for differential diagnosis depending on phosphorylation sites.

• ApoE has no effect on CSF-tTau and pTau. Kinesin polymorphism is associated with high CSF-pTau levels.

• CSF-pTau levels are altered very early during the disease course of AD and are therefore useful predictive biomarkers.

• CSF-tTau levels has a diagnostic value in AD and CJD and a prognostic value in transient acute neuronal damage syndroms.

• The combination of CSF Tau levels together or with other biomarkers increases sensitivity and specificity of diagnosis.

• Stable levels of CSF tTau and pTau are potentially useful for monitoring novel therapeutic assays.

• The new identified multiple fragments of Tau in brain and CSF are promising candidates for differential diagnosis of Tauopathies.

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References:

- 1 . Buee L , Bussiere T , Buee-Scherrer V , Delacourte A , Hof PR . Tau protein isoforms, phosphorylation and role in neurodegenerative disorders . Brain Res Brain Res Rev . 2000 ; 33 : (1) 95 - 130
- 2 . Stefansson H , Helgason A , Thorleifsson G , Steinthorsdottir V , Masson G , Barnard J , Baker A , Jonasdottir A , Ingason A , Gudnadottir VG . A common inversion under selection in Europeans . Nature genetics . 2005 ; 37 : (2) 129 - 137
- 3 . Schraen-Maschke S , Dhaenens CM , Delacourte A , Sablonniere B . Microtubule-associated protein tau gene: a risk factor in human neurodegenerative diseases . Neurobiology of disease . 2004 ; 15 : (3) 449 - 460
- 4 . Pittman AM , Myers AJ , Duckworth J , Bryden L , Hanson M , Abou-Sleiman P , Wood NW , Hardy J , Lees A , de Silva R . The structure of the tau haplotype in controls and in progressive supranuclear palsy . Human molecular genetics . 2004 ; 13 : (12) 1267 - 1274
- 5 . Andreadis A . Tau gene alternative splicing: expression patterns, regulation and modulation of function in normal brain and neurodegenerative diseases . Biochim Biophys Acta . 2005 ; 1739 : (2-3) 91 - 103
- 6 . Gallo JM , Noble W , Martin TR . RNA and protein-dependent mechanisms in tauopathies: consequences for therapeutic strategies . Cell Mol Life Sci . 2007 ; 64 : (13) 1701 - 1714
- 7 . Wang JZ , Liu F . Microtubule-associated protein tau in development, degeneration and protection of neurons . Progress in neurobiology . 2008 ; 85 : (2) 148 - 175
- 8 . Utton MA , Noble WJ , Hill JE , Anderton BH , Hanger DP . Molecular motors implicated in the axonal transport of tau and alpha-synuclein . Journal of cell science . 2005 ; 118 : (Pt 20) 4645 - 4654
- 9 . Magnani E , Fan J , Gasparini L , Golding M , Williams M , Schiavo G , Goedert M , Amos LA , Spillantini MG . Interaction of tau protein with the dynactin complex . The EMBO journal . 2007 ; 26 : (21) 4546 - 4554
- 10 . Reynolds CH , Garwood CJ , Wray S , Price C , Kellie S , Perera T , Zvelebil M , Yang A , Sheppard PW , Varndell IM . Phosphorylation regulates tau interactions with SH3 domains of phosphatidylinositol-3-kinase, phospholipase cgamma 1, GRB2 and SRC-family kinases . The Journal of biological chemistry . 2008 ;
- 11 . Deshpande A , Win KM , Busciglio J . Tau isoform expression and regulation in human cortical neurons . Faseb J . 2008 ;
- 12 . Goedert M , Spillantini MG , Jakes R , Rutherford D , Crowther RA . Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease . Neuron . 1989 ; 3 : (4) 519 - 526
- 13 . Hanger DP , Byers HL , Wray S , Leung KY , Saxton MJ , Seereeram A , Reynolds CH , Ward MA , Anderton BH . Novel phosphorylation sites in tau from Alzheimer brain support a role for casein kinase 1 in disease pathogenesis . The Journal of biological chemistry . 2007 ; 282 : (32) 23645 - 23654
- 14 . Sergeant N , Bretteville A , Hamdane M , Caillet-Boudin ML , Grognet P , Bombois S , Blum D , Delacourte A , Pasquier F , Vanmechelen E . Biochemistry of Tau in Alzheimer's disease and related neurological disorders . Expert Rev Proteomics . 2008 ; 5 : (2) 207 - 224
- 15 . Sergeant N , Delacourte A , Buee L . Tau protein as a differential biomarker of tauopathies . Biochim Biophys Acta . 2005 ; 1739 : (2-3) 179 - 197
- 16 . Mandelkow EM , Thies E , Trinczek B , Biernat J , Mandelkow E . MARK/PAR1 kinase is a regulator of microtubule-dependent transport in axons . The Journal of cell biology . 2004 ; 167 : (1) 99 - 110
- 17 . Lee G , Thangavel R , Sharma VM , Litersky JM , Bhaskar K , Fang SM , Do LH , Andreadis A , Van Hoesen G , Ksiezak-Reding H . Phosphorylation of tau by fyn: implications for Alzheimer's disease . J Neurosci . 2004 ; 24 : (9) 2304 - 2312
- 18 . Williamson R , Scales T , Clark BR , Gibb G , Reynolds CH , Kellie S , Bird IN , Varndell IM , Sheppard PW , Everall I . Rapid tyrosine phosphorylation of neuronal proteins including tau and focal adhesion kinase in response to amyloid-beta peptide exposure: involvement of Src family protein kinases . J Neurosci . 2002 ; 22 : (1) 10 - 20
- 19 . Derkinderen P , Scales TM , Hanger DP , Leung KY , Byers HL , Ward MA , Lenz C , Price C , Bird IN , Perera T . Tyrosine 394 is phosphorylated in Alzheimer's paired helical filament tau and in fetal tau with c-Abl as the candidate tyrosine kinase . J Neurosci . 2005 ; 25 : (28) 6584 - 6593
- 20 . Koolen DA , Vissers LE , Pfundt R , de Leeuw N , Knight SJ , Regan R , Kooy RF , Reyniers E , Romano C , Fichera M . A new chromosome 17q21.31 microdeletion syndrome associated with a common inversion polymorphism . Nature genetics . 2006 ; 38 : (9) 999 - 1001
- 21 . Sharp AJ , Hansen S , Selzer RR , Cheng Z , Regan R , Hurst JA , Stewart H , Price SM , Blair E , Hennekam RC . Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome . Nature genetics . 2006 ; 38 : (9) 1038 - 1042
- 22 . Varela MC , Krepischi-Santos AC , Paz JA , Knijnenburg J , Szuhai K , Rosenberg C , Koiffmann CP . A 17q21.31 microdeletion encompassing the MAPT gene in a mentally impaired patient . Cytogenetic and genome research . 2006 ; 114 : (1) 89 - 92
- 23 . Shaw-Smith C , Pittman AM , Willatt L , Martin H , Rickman L , Gribble S , Curley R , Cumming S , Dunn C , Kalaitzopoulos D . Microdeletion encompassing MAPT at chromosome 17q21.3 is associated with developmental delay and learning disability . Nature genetics . 2006 ; 38 : (9) 1032 - 1037
- 24 . Kirchhoff M , Bisgaard AM , Duno M , Hansen FJ , Schwartz M . A 17q21.31 microduplication, reciprocal to the newly described 17q21.31 microdeletion, in a girl with severe psychomotor developmental delay and dysmorphic craniofacial features . European journal of medical genetics . 2007 ; 50 : (4) 256 - 263
- 25 . Wszolek ZK , Tsuboi Y , Ghetti B , Pickering-Brown S , Baba Y , Cheshire WP . Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) . Orphanet journal of rare diseases . 2006 ; 1 : 30 -
- 26 . Boeve BF , Hutton M . Refining frontotemporal dementia with parkinsonism linked to chromosome 17: introducing FTDP-17 (MAPT) and FTDP-17 (PGRN) . Archives of neurology . 2008 ; 65 : (4) 460 - 464
- 27 . Caffrey TM , Wade-Martins R . Functional MAPT haplotypes: bridging the gap between genotype and neuropathology . Neurobiology of disease . 2007 ; 27 : (1) 1 - 10
- 28 . Zabetian CP , Hutter CM , Factor SA , Nutt JG , Higgins DS , Griffith A , Roberts JW , Leis BC , Kay DM , Yearout D . Association analysis of MAPT H1 haplotype and subhaplotypes in Parkinson's disease . Annals of neurology . 2007 ; 62 : (2) 137 - 144
- 29 . Pittman AM , Myers AJ , Abou-Sleiman P , Fung HC , Kaleem M , Marlowe L , Duckworth J , Leung D , Williams D , Kilford L . Linkage disequilibrium fine mapping and haplotype association analysis of the tau gene in progressive supranuclear palsy and corticobasal degeneration . Journal of medical genetics . 2005 ; 42 : (11) 837 - 846
- 30 . Myers AJ , Kaleem M , Marlowe L , Pittman AM , Lees AJ , Fung HC , Duckworth J , Leung D , Gibson A , Morris CM . The H1c haplotype at the MAPT locus is associated with Alzheimer's disease . Human molecular genetics . 2005 ; 14 : (16) 2399 - 2404
- 31 . Mukherjee O , Pastor P , Cairns NJ , Chakraverty S , Kauwe JS , Shears S , Behrens MI , Budde J , Hinrichs AL , Norton J . HDDD2 is a familial frontotemporal lobar degeneration with ubiquitin-positive, tau-negative inclusions caused by a missense mutation in the signal peptide of progranulin . Annals of neurology . 2006 ; 60 : (3) 314 - 322
- 32 . Rademakers R , Melquist S , Cruts M , Theuns J , Del-Favero J , Poorkaj P , Baker M , Sleegers K , Crook R , De Pooter T . High-density SNP haplotyping suggests altered regulation of tau gene expression in progressive supranuclear palsy . Human molecular genetics . 2005 ; 14 : (21) 3281 - 3292

- 33 . Hayesmoore JB , Bray NJ , Cross WC , Owen MJ , O'Donovan MC , Morris HR . The effect of age and the H1c MAPT haplotype on MAPT expression in human brain . Neurobiology of aging . 2008 ;
- 34 . Myers AJ , Pittman AM , Zhao AS , Rohrer K , Kaleem M , Marlowe L , Lees A , Leung D , McKeith IG , Perry RH . The MAPT H1c risk haplotype is associated with increased expression of tau and especially of 4 repeat containing transcripts . Neurobiology of disease . 2007 ; 25 : (3) 561 - 570
- 35 . Caffrey TM , Joachim C , Paracchini S , Esiri MM , Wade-Martins R . Haplotype-specific expression of exon 10 at the human MAPT locus . Human molecular genetics . 2006 ; 15 : (24) 3529 - 3537
- 36 . Llado A , Ezquerra M , Gaig C , Sanchez-Valle R , Tolosa E , Molinuevo JL . Brain tau expression and correlation with the H1/H1 tau genotype in frontotemporal lobar degeneration patients . J Neural Transm . 2007 ; 114 : (12) 1585 - 1588
- 37 . Goris A , Williams-Gray CH , Clark GR , Foltynie T , Lewis SJ , Brown J , Ban M , Spillantini MG , Compston A , Burn DJ . Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease . Annals of neurology . 2007 ; 62 : (2) 145 - 153
- 38 . Meola G , Sansone V . Cerebral involvement in myotonic dystrophies . Muscle & nerve . 2007 ; 36 : (3) 294 - 306
- 39 . Sansone V , Gandossini S , Cotelli M , Calabria M , Zanetti O , Meola G . Cognitive impairment in adult myotonic dystrophies: a longitudinal study . Neurol Sci . 2007 ; 28 : (1) 9 - 15
- 40 . Ranum LP , Cooper TA . RNA-mediated neuromuscular disorders . Annual review of neuroscience . 2006 ; 29 : 259 - 277
- 41 . Vermersch P , Sergeant N , Ruchoux MM , Hofmann-Radvanyi H , Wattez A , Petit H , Dwailly P , Delacourte A . Specific tau variants in the brains of patients with myotonic dystrophy . Neurology . 1996 ; 47 : (3) 711 - 717
- 42 . Kuyumcu-Martinez NM , Cooper TA . Misregulation of alternative splicing causes pathogenesis in myotonic dystrophy . Progress in molecular and subcellular biology . 2006 ; 44 : 133 - 159
- 43 . Jiang H , Mankodi A , Swanson MS , Moxley RT , Thornton CA . Myotonic dystrophy type 1 is associated with nuclear foci of mutant RNA, sequestration of muscleblind proteins and deregulated alternative splicing in neurons . Human molecular genetics . 2004 ; 13 : (24) 3079 - 3088
- 44 . Sergeant N , Sablonniere B , Schraen-Maschke S , Ghestem A , Maurage CA , Wattez A , Vermersch P , Delacourte A . Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1 . Human molecular genetics . 2001 ; 10 : (19) 2143 - 2155
- 45 . Maurage CA , Udd B , Ruchoux MM , Vermersch P , Kalimo H , Krahe R , Delacourte A , Sergeant N . Similar brain tau pathology in DM2/PROMM and DM1/Steinert disease . Neurology . 2005 ; 65 : (10) 1636 - 1638
- 46 . Dhaenens CM , Schraen-Maschke S , Tran H , Vingtdeux V , Ghanem D , Leroy O , Delplanque J , Vanbrussel E , Delacourte A , Vermersch P . Overexpression of MBNL1 fetal isoforms and modified splicing of Tau in the DM1 brain: two individual consequences of CUG trinucleotide repeats . Experimental neurology . 2008 ; 210 : (2) 467 - 478
- 47 . Cho DH , Tapscott SJ . Myotonic dystrophy: emerging mechanisms for DM1 and DM2 . Biochim Biophys Acta . 2007 ; 1772 : (2) 195 - 204
- 48 . Delobel P , Mailliot C , Hamdane M , Sambo AV , Begard S , Violleau A , Delacourte A , Buee L . Stable-tau overexpression in human neuroblastoma cells: an open door for explaining neuronal death in tauopathies . Annals of the New York Academy of Sciences . 2003 ; 1010 : 623 - 634
- 49 . Higuchi M , Ishihara T , Zhang B , Hong M , Andreadis A , Trojanowski J , Lee VM . Transgenic mouse model of tauopathies with glial pathology and nervous system degeneration . Neuron . 2002 ; 35 : (3) 433 - 446
- 50 . Ingelsson M , Ramasamy K , Russ C , Freeman SH , Orne J , Raju S , Matsui T , Growdon JH , Frosch MP , Ghetti B . Increase in the relative expression of tau with four microtubule binding repeat regions in frontotemporal lobar degeneration and progressive supranuclear palsy brains . Acta neuropathologica . 2007 ; 114 : (5) 471 - 479
- 51 . Ohkubo T , Sakasegawa Y , Toda H , Kishida H , Arima K , Yamada M , Takahashi H , Mizusawa H , Hachiya NS , Kaneko K . Three-repeat Tau 69 is a major tau isoform in laser-microdissected Pick bodies . Amyloid . 2006 ; 13 : (1) 1 - 5
- 52 . Tobin JE , Latourelle JC , Lew MF , Klein C , Suchowersky O , Shill HA , Golbe LI , Mark MH , Growdon JH , Wooten GF . Haplotypes and gene expression implicate the MAPT region for Parkinson disease: the GenePD Study . Neurology . 2008 ; 71 : (1) 28 - 34
- 53 . Glatz DC , Rujescu D , Tang Y , Berendt FJ , Hartmann AM , Faltraco F , Rosenberg C , Hulette C , Jellinger K , Hampel H . The alternative splicing of tau exon 10 and its regulatory proteins CLK2 and TRA2-BETA1 changes in sporadic Alzheimer's disease . Journal of neurochemistry . 2006 ; 96 : (3) 635 - 644
- 54 . Arai T , Ikeda K , Akiyama H , Tsuchiya K , Iritani S , Ishiguro K , Yagishita S , Oda T , Odawara T , Iseki E . Different immunoreactivities of the microtubule-binding region of tau and its molecular basis in brains from patients with Alzheimer's disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration . Acta neuropathologica . 2003 ; 105 : (5) 489 - 498
- 55 . Umeda Y , Taniguchi S , Arima K , Piao YS , Takahashi H , Iwatsubo T , Mann D , Hasegawa M . Alterations in human tau transcripts correlate with those of neurofilament in sporadic tauopathies . Neuroscience letters . 2004 ; 359 : (3) 151 - 154
- 56 . Ingelsson M , Ramasamy K , Cantuti-Castelvetri I , Skoglund L , Matsui T , Orne J , Kowa H , Raju S , Vanderburg CR , Augustinack JC . No alteration in tau exon 10 alternative splicing in tangle-bearing neurons of the Alzheimer's disease brain . Acta neuropathologica . 2006 ; 112 : (4) 439 - 449
- 57 . Hernandez F , Avila J . Tauopathies . Cell Mol Life Sci . 2007 ; 64 : (17) 2219 - 2233
- 58 . Andorfer C , Acker CM , Kress Y , Hof PR , Duff K , Davies P . Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms . J Neurosci . 2005 ; 25 : (22) 5446 - 5454
- 59 . Santacruz K , Lewis J , Spires T , Paulson J , Kotilinek L , Ingelsson M , Guimaraes A , DeTure M , Ramsden M , McGowan E . Tau suppression in a neurodegenerative mouse model improves memory function . Science (New York, NY) . 2005 ; 309 : (5733) 476 - 481
- 60 . Lee HG , Perry G , Moreira PI , Garrett MR , Liu Q , Zhu X , Takeda A , Nunomura A , Smith MA . Tau phosphorylation in Alzheimer's disease: pathogen or protector? . Trends in molecular medicine . 2005 ; 11 : (4) 164 - 169
- 61 . Arrasate M , Mitra S , Schweitzer ES , Segal MR , Finkbeiner S . Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death . Nature . 2004 ; 431 : (7010) 805 - 810
- 62 . Wittmann CW , Wszolek MF , Shulman JM , Salvaterra PM , Lewis J , Hutton M , Feany MB . Tauopathy in Drosophila: neurodegeneration without neurofibrillary tangles . Science (New York, NY) . 2001 ; 293 : (5530) 711 - 714
- 63 . Terwel D , Lasrado R , Snauwaert J , Vandeweert E , Van Haesendonck C , Borghgraef P , Van Leuven F . Changed conformation of mutant Tau-P301L underlies the moribund tauopathy, absent in progressive, nonlethal axonopathy of Tau-4R/2N transgenic mice . The Journal of biological chemistry . 2005 ; 280 : (5) 3963 - 3973
- 64 . Chen S , Li B , Grundke-Iqbal I , Iqbal K . I1PP2A affects tau phosphorylation via association with the catalytic subunit of protein phosphatase 2A . The Journal of biological chemistry . 2008 ; 283 : (16) 10513 - 10521
- 65 . Augustinack JC , Schneider A , Mandelkow EM , Hyman BT . Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease . Acta neuropathologica . 2002 ; 103 : (1) 26 - 35
- 66 . Yoshida H , Goedert M . Sequential phosphorylation of tau protein by cAMP-dependent protein kinase and SAPK4/p38delta or JNK2 in the presence of heparin generates the AT100 epitope . Journal of neurochemistry . 2006 ; 99 : (1) 154 - 164
- 67 . Delacourte A , Robitaille Y , Sergeant N , Buee L , Hof PR , Wattez A , Laroche-Cholette A , Mathieu J , Chagnon P , Gauvreau D . Specific pathological Tau protein variants characterize Pick's disease . Journal of neuropathology and experimental neurology . 1996 ; 55 : (2) 159 - 168
- 68 . Probst A , Tolnay M , Langui D , Goedert M , Spillantini MG . Pick's disease: hyperphosphorylated tau protein segregates to the somatoaxonal compartment . Acta neuropathologica . 1996 ; 92 : (6) 588 - 596
- 69 . Schindowski K , Bretteville A , Leroy K , Begard S , Brion JP , Hamdane M , Buee L . Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits . The American journal of pathology . 2006 ; 169 : (2) 599 - 616
- 70 . Delacourte A , David JP , Sergeant N , Buee L , Wattez A , Vermersch P , Ghozali F , Fallet-Bianco C , Pasquier F , Lebert F . The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease . Neurology . 1999 ; 52 : (6) 1158 - 1165
- 71 . Shimura H , Schwartz D , Gygi SP , Kosik KS . CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival . The Journal of biological chemistry . 2004 ; 279 : (6) 4869 - 4876

- 72 . Fasulo L , Ugolini G , Visintin M , Bradbury A , Brancolini C , Verzillo V , Novak M , Cattaneo A . The neuronal microtubule-associated protein tau is a substrate for caspase-3 and an effector of apoptosis . Journal of neurochemistry . 2000 ; 75 : (2) 624 - 633
- 73 . Gamblin TC , Chen F , Zambrano A , Abraha A , Lagalwar S , Guillozet AL , Lu M , Fu Y , Garcia-Sierra F , LaPointe N . Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease . Proceedings of the National Academy of Sciences of the United States of America . 2003 ; 100 : (17) 10032 - 10037
- 74 . Yang LS , Ksiezak-Reding H . Calpain-induced proteolysis of normal human tau and tau associated with paired helical filaments . European journal of biochemistry / FEBS . 1995 ; 233 : (1) 9 - 17
- 75 . David DC , Layfield R , Serpell L , Narain Y , Goedert M , Spillantini MG . Proteasomal degradation of tau protein . Journal of neurochemistry . 2002 ; 83 : (1) 176 - 185
- 76 . Guillozet-Bongaarts AL , Cahill ME , Cryns VL , Reynolds MR , Berry RW , Binder LI . Pseudophosphorylation of tau at serine 422 inhibits caspase cleavage: in vitro evidence and implications for tangle formation in vivo . Journal of neurochemistry . 2006 ; 97 : (4) 1005 - 1014
- 77 . Fasulo L , Ugolini G , Cattaneo A . Apoptotic effect of caspase-3 cleaved tau in hippocampal neurons and its potentiation by tau FTDP-mutation N279K . J Alzheimers Dis . 2005 ; 7 : (1) 3 - 13
- 78 . Albrecht S , Bourdeau M , Bennett D , Mufson EJ , Bhattacharjee M , LeBlanc AC . Activation of caspase-6 in aging and mild cognitive impairment . The American journal of pathology . 2007 ; 170 : (4) 1200 - 1209
- 79 . Delobel P , Leroy O , Hamdane M , Sambo AV , Delacourte A , Buee L . Proteasome inhibition and Tau proteolysis: an unexpected regulation . FEBS letters . 2005 ; 579 : (1) 1 - 5
- 80 . Cotman CW , Poon WW , Rissman RA , Blurton-Jones M . The role of caspase cleavage of tau in Alzheimer disease neuropathology . Journal of neuropathology and experimental neurology . 2005 ; 64 : (2) 104 - 112
- 81 . Garcia-Sierra F , Ghoshal N , Quinn B , Berry RW , Binder LI . Conformational changes and truncation of tau protein during tangle evolution in Alzheimer's disease . J Alzheimers Dis . 2003 ; 5 : (2) 65 - 77
- 82 . Wray S , Saxton M , Anderton BH , Hanger DP . Direct analysis of tau from PSP brain identifies new phosphorylation sites and a major fragment of N-terminally cleaved tau containing four microtubule-binding repeats . Journal of neurochemistry . 2008 ;
- 83 . Delacourte A , Sergeant N , Buee L . Neurodegenerative diseases: Tau proteins in neurodegenerative diseases other than Alzheimer's disease . Humana Press ; 2007 ; 690 -
- 84 . Hanger DP , Mann DM , Neary D , Anderton BH . Molecular pathology of Alzheimer's disease in sporadic and familial Alzheimer's disease with mutations in the amyloid precursor protein . Biochemical Society transactions . 1992 ; 20 : (3) 642 - 645
- 85 . Sergeant N , David JP , Lefranc D , Vermersch P , Wattez A , Delacourte A . Different distribution of phosphorylated tau protein isoforms in Alzheimer's and Pick's diseases . FEBS letters . 1997 ; 412 : (3) 578 - 582
- 86 . Goedert M , Spillantini MG , Cairns NJ , Crowther RA . Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms . Neuron . 1992 ; 8 : (1) 159 - 168
- 87 . Sergeant N , Wattez A , Delacourte A . Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: tau pathologies with exclusively “ exon 10” isoforms . Journal of neurochemistry . 1999 ; 72 : (3) 1243 - 1249
- 88 . Tolnay M , Sergeant N , Ghestem A , Chalbot S , De Vos RA , Jansen Steur EN , Probst A , Delacourte A . Argyrophilic grain disease and Alzheimer's disease are distinguished by their different distribution of tau protein isoforms . Acta neuropathologica . 2002 ; 104 : (4) 425 - 434
- 89 . Cairns NJ , Bigio EH , Mackenzie IR , Neumann M , Lee VM , Hatanpaa KJ , White CL 3rd , Schneider JA , Grinberg LT , Halliday G . Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration . Acta neuropathologica . 2007 ; 114 : (1) 5 - 22
- 90 . Hickey C , Chisholm T , Passmore MJ , O'Brien JD , Johnston J . Differentiating the dementias. Revisiting synucleinopathies and tauopathies . Current Alzheimer research . 2008 ; 5 : (1) 52 - 60
- 91 . Buerger K , Zinkowski R , Teipel SJ , Arai H , DeBernardis J , Kerkman D , McCulloch C , Padberg F , Faltraco F , Goernitz A . Differentiation of geriatric major depression from Alzheimer's disease with CSF tau protein phosphorylated at threonine 231 . The American journal of psychiatry . 2003 ; 160 : (2) 376 - 379
- 92 . Blennow K . Cerebrospinal fluid protein biomarkers for Alzheimer's disease . NeuroRx . 2004 ; 1 : (2) 213 - 225
- 93 . Blennow K , Vanmechelen E . CSF markers for pathogenic processes in Alzheimer's disease: diagnostic implications and use in clinical neurochemistry . Brain research bulletin . 2003 ; 61 : (3) 235 - 242
- 94 . Sjogren M , Vanderstichele H , Agren H , Zachrisson O , Edsbagge M , Wikkelso C , Skoog I , Wallin A , Wahlund LO , Marcusson J . Tau and Abeta42 in cerebrospinal fluid from healthy adults 21-93 years of age: establishment of reference values . Clinical chemistry . 2001 ; 47 : (10) 1776 - 1781
- 95 . Clark CM , Xie S , Chittams J , Ewbank D , Peskind E , Galasko D , Morris JC , McKeel DW Jr , Farlow M , Weitlauf SL . Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? . Archives of neurology . 2003 ; 60 : (12) 1696 - 1702
- 96 . Sanchez-Juan P , Green A , Ladogana A , Cuadrado-Corrales N , Saanchez-Valle R , Mitrovaa E , Stoeck K , Sklaviadis T , Kulczycki J , Hess K . CSF tests in the differential diagnosis of Creutzfeldt-Jakob disease . Neurology . 2006 ; 67 : (4) 637 - 643
- 97 . Goodall CA , Head MW , Everington D , Ironside JW , Knight RS , Green AJ . Raised CSF phospho-tau concentrations in variant Creutzfeldt-Jakob disease: diagnostic and pathological implications . Journal of neurology, neurosurgery, and psychiatry . 2006 ; 77 : (1) 89 - 91
- 98 . Medana IM , Lindert RB , Wurster U , Hien TT , Day NP , Phu NH , Mai NT , Chuong LV , Chau TT , Turner GD . Cerebrospinal fluid levels of markers of brain parenchymal damage in Vietnamese adults with severe malaria . Transactions of the Royal Society of Tropical Medicine and Hygiene . 2005 ; 99 : (8) 610 - 617
- 99 . Matsushita S , Miyakawa T , Maesato H , Matsui T , Yokoyama A , Arai H , Higuchi S , Kashima H . Elevated cerebrospinal fluid tau protein levels in Wernicke's encephalopathy . Alcoholism, clinical and experimental research . 2008 ; 32 : (6) 1091 - 1095
- 100 . de Bont JM , Vanderstichele H , Reddingius RE , Pieters R , van Gool SW . Increased total-Tau levels in cerebrospinal fluid of pediatric hydrocephalus and brain tumor patients . Eur J Paediatr Neurol . 2008 ; 12 : (4) 334 - 341
- 101 . Bian H , Van Swieten JC , Leight S , Massimo L , Wood E , Forman M , Moore P , de Koning I , Clark CM , Rosso S . CSF biomarkers in frontotemporal lobar degeneration with known pathology . Neurology . 2008 ; 70 : (19 Pt 2) 1827 - 1835
- 102 . Grossman M , Farmer J , Leight S , Work M , Moore P , Van Deerlin V , Pratico D , Clark CM , Coslett HB , Chatterjee A . Cerebrospinal fluid profile in frontotemporal dementia and Alzheimer's disease . Annals of neurology . 2005 ; 57 : (5) 721 - 729
- 103 . Pijnenburg YA , Schoonenboom NS , Rosso SM , Mulder C , Van Kamp GJ , Van Swieten JC , Scheltens P . CSF tau and Abeta42 are not useful in the diagnosis of frontotemporal lobar degeneration . Neurology . 2004 ; 62 : (9) 1649 -
- 104 . Parnetti L , Tiraboschi P , Lanari A , Peducci M , Padiglioni C , D'Amore C , Pierguidi L , Tambasco N , Rossi A , Calabresi P . Cerebrospinal Fluid Biomarkers in Parkinson's Disease with Dementia and Dementia with Lewy Bodies . Biological psychiatry . 2008 ;
- 105 . Mollenhauer B , Cepek L , Bibl M , Wiltfang J , Schulz-Schaeffer WJ , Ciesielczyk B , Neumann M , Steinacker P , Kretzschmar HA , Poser S . Tau protein, Abeta42 and S-100B protein in cerebrospinal fluid of patients with dementia with Lewy bodies . Dementia and geriatric cognitive disorders . 2005 ; 19 : (2-3) 164 - 170
- 106 . Bibl M , Mollenhauer B , Esselmann H , Lewczuk P , Trenkwalder C , Brechlin P , Ruther E , Kornhuber J , Otto M , Wiltfang J . CSF diagnosis of Alzheimer's disease and dementia with Lewy bodies . J Neural Transm . 2006 ; 113 : (11) 1771 - 1778
- 107 . Tschampa HJ , Schulz-Schaeffer W , Wiltfang J , Poser S , Otto M , Neumann M , Kretzschmar HA . Decreased CSF amyloid beta42 and normal tau levels in dementia with Lewy bodies . Neurology . 2001 ; 56 : (4) 576 -
- 108 . Mollenhauer B , Trenkwalder C , von Ahsen N , Bibl M , Steinacker P , Brechlin P , Schindehuette J , Poser S , Wiltfang J , Otto M . Beta-amlyoid 1-42 and tau-protein in cerebrospinal fluid of patients with Parkinson's disease dementia . Dementia and geriatric cognitive disorders . 2006 ; 22 : (3) 200 - 208
- 109 . Borroni B , Gardoni F , Parnetti L , Magno L , Malinverno M , Saggese E , Calabresi P , Spillantini MG , Padovani A , Di Luca M . Pattern of Tau forms in CSF is altered in progressive supranuclear palsy . Neurobiology of aging . 2007 ;
- 110 . Brettschneider J , Petzold A , Sussmuth SD , Ludolph AC , Tumani H . Axonal damage markers in cerebrospinal fluid are increased in ALS . Neurology . 2006 ; 66 : (6) 852 - 856

- 111 . Jimenez-Jimenez FJ , Hernanz A , Medina-Acebron S , de Bustos F , Zurdo JM , Alonso H , Puertas I , Barcenilla B , Sayed Y , Cabrera-Valdivia F . Tau protein concentrations in cerebrospinal fluid of patients with amyotrophic lateral sclerosis . Acta neurologica Scandinavica . 2005 ; 111 : (2) 114 - 117
- 112 . Boban M , Grbic K , Mladinov M , Hof PR , Sussmair C , Ackl N , Stanic G , Bader B , Danek A , Simic G . Cerebrospinal fluid markers in differential diagnosis of Alzheimer's disease and vascular dementia . Collegium antropologicum . 2008 ; 32 : (Suppl 1) 31 - 36
- 113 . Hein Nee Maier K , Kohler A , Diem R , Sattler MB , Demmer I , Lange P , Bahr M , Otto M . Biological markers for axonal degeneration in CSF and blood of patients with the first event indicative for multiple sclerosis . Neuroscience letters . 2008 ; 436 : (1) 72 - 76
- 114 . Brettschneider J , Maier M , Arda S , Claus A , Sussmuth SD , Kassubek J , Tumani H . Tau protein level in cerebrospinal fluid is increased in patients with early multiple sclerosis . Multiple sclerosis (Houndmills, Basingstoke, England) . 2005 ; 11 : (3) 261 - 265
- 115 . Blennow K . CSF biomarkers for Alzheimer's disease: use in early diagnosis and evaluation of drug treatment . Expert review of molecular diagnostics . 2005 ; 5 : (5) 661 - 672
- 116 . Hampel H , Buerger K , Zinkowski R , Teipel SJ , Goernitz A , Andreasen N , Sjoegren M , DeBernardis J , Kerkman D , Ishiguro K . Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study . Archives of general psychiatry . 2004 ; 61 : (1) 95 - 102
- 117 . Riemenschneider M , Wagenpfeil S , Vanderstichele H , Otto M , Wiltfang J , Kretzschmar H , Vanmechelen E , Forstl H , Kurz A . Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias . Molecular psychiatry . 2003 ; 8 : (3) 343 - 347
- 118 . Sjogren M , Davidsson P , Wallin A , Granerus AK , Grundstrom E , Askmark H , Vanmechelen E , Blennow K . Decreased CSF-beta-amyloid 42 in Alzheimer's disease and amyotrophic lateral sclerosis may reflect mismetabolism of beta-amyloid induced by disparate mechanisms . Dementia and geriatric cognitive disorders . 2002 ; 13 : (2) 112 - 118
- 119 . Buerger K , Zinkowski R , Teipel SJ , Tapiola T , Arai H , Blennow K , Andreasen N , Hofmann-Kiefer K , DeBernardis J , Kerkman D . Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of tau protein phosphorylated at threonine 231 . Archives of neurology . 2002 ; 59 : (8) 1267 - 1272
- 120 . Vanderstichele H , De Vreese K , Blennow K , Andreasen N , Sindic C , Ivanoiu A , Hampel H , Burger K , Parnetti L , Lanari A . Analytical performance and clinical utility of the INNOTEST PHOSPHO-TAU181P assay for discrimination between Alzheimer's disease and dementia with Lewy bodies . Clin Chem Lab Med . 2006 ; 44 : (12) 1472 - 1480
- 121 . Verbeek MM , Pijnenburg YA , Schoonenboom NS , Kremer BP , Scheltens P . Cerebrospinal fluid tau levels in frontotemporal dementia . Annals of neurology . 2005 ; 58 : (4) 656 - 657 author reply 657
- 122 . Lewczuk P , Esselmann H , Otto M , Maler JM , Henkel AW , Henkel MK , Eikenberg O , Antz C , Krause WR , Reulbach U . Neurochemical diagnosis of Alzheimer's dementia by CSF Abeta42, Abeta42/Abeta40 ratio and total tau . Neurobiology of aging . 2004 ; 25 : (3) 273 - 281
- 123 . Huey ED , Mirza N , Putnam KT , Soares H , Csako G , Levy JA , Copenhaver B , Cohen RM , Sunderland T . Stability of CSF beta-amyloid(1-42) and tau levels by APOE genotype in Alzheimer patients . Dementia and geriatric cognitive disorders . 2006 ; 22 : (1) 48 - 53
- 124 . Blomqvist ME , Reynolds C , Katzov H , Feuk L , Andreasen N , Bogdanovic N , Blennow K , Brookes AJ , Prince JA . Towards compendia of negative genetic association studies: an example for Alzheimer disease . Human genetics . 2006 ; 119 : (1- 2) 29 - 37
- 125 . Johansson A , Zetterberg H , Hakansson A , Nissbrandt H , Blennow K . TAU haplotype and the Saitohin Q7R gene polymorphism do not influence CSF Tau in Alzheimer's disease and are not associated with frontotemporal dementia or Parkinson's disease . Neuro-degenerative diseases . 2005 ; 2 : (1) 28 - 35
- 126 . Kauwe JS , Cruchaga C , Mayo K , Fenoglio C , Bertelsen S , Nowotny P , Galimberti D , Scarpini E , Morris JC , Fagan AM . Variation in MAPT is associated with cerebrospinal fluid tau levels in the presence of amyloid-beta deposition . Proceedings of the National Academy of Sciences of the United States of America . 2008 ; 105 : (23) 8050 - 8054
- 127 . Laws SM , Friedrich P , Diehl-Schmid J , Muller J , Eisele T , Bauml J , Forstl H , Kurz A , Riemenschneider M . Fine mapping of the MAPT locus using quantitative trait analysis identifies possible causal variants in Alzheimer's disease . Molecular psychiatry . 2007 ; 12 : (5) 510 - 517
- 128 . Dhaenens CM , Van Brussel E , Schraen-Maschke S , Pasquier F , Delacourte A , Sablonniere B . Association study of three polymorphisms of kinesin light-chain 1 gene with Alzheimer's disease . Neuroscience letters . 2004 ; 368 : (3) 290 - 292
- 129 . Andersson ME , Sjolander A , Andreasen N , Minthon L , Hansson O , Bogdanovic N , Jern C , Jood K , Wallin A , Blennow K . Kinesin gene variability may affect tau phosphorylation in early Alzheimer's disease . International journal of molecular medicine . 2007 ; 20 : (2) 233 - 239
- 130 . Dubois B , Feldman HH , Jacova C , Dekosky ST , Barberger-Gateau P , Cummings J , Delacourte A , Galasko D , Gauthier S , Jicha G . Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria . Lancet neurology . 2007 ; 6 : (8) 734 - 746
- 131 . Andreasen N , Blennow K . CSF biomarkers for mild cognitive impairment and early Alzheimer's disease . Clinical neurology and neurosurgery . 2005 ; 107 : (3) 165 - 173
- 132 . Andreasen N , Vanmechelen E , Vanderstichele H , Davidsson P , Blennow K . Cerebrospinal fluid levels of total-tau, phospho-tau and A beta 42 predicts development of Alzheimer's disease in patients with mild cognitive impairment . Acta Neurol Scand Suppl . 2003 ; 179 : 47 - 51
- 133 . Ewers M , Buerger K , Teipel SJ , Scheltens P , Schroder J , Zinkowski RP , Bouwman FH , Schonknecht P , Schoonenboom NS , Andreasen N . Multicenter assessment of CSF-phosphorylated tau for the prediction of conversion of MCI . Neurology . 2007 ; 69 : (24) 2205 - 2212
- 134 . Buerger K , Teipel SJ , Zinkowski R , Blennow K , Arai H , Engel R , Hofmann-Kiefer K , McCulloch C , Ptok U , Heun R . CSF tau protein phosphorylated at threonine 231 correlates with cognitive decline in MCI subjects . Neurology . 2002 ; 59 : (4) 627 - 629
- 135 . de Leon MJ , DeSanti S , Zinkowski R , Mehta PD , Pratico D , Segal S , Rusinek H , Li J , Tsui W , Saint Louis LA . Longitudinal CSF and MRI biomarkers improve the diagnosis of mild cognitive impairment . Neurobiology of aging . 2006 ; 27 : (3) 394 - 401
- 136 . Hansson O , Zetterberg H , Buchhave P , Londos E , Blennow K , Minthon L . Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study . Lancet neurology . 2006 ; 5 : (3) 228 - 234
- 137 . Herukka SK , Hallikainen M , Soininen H , Pirttila T . CSF Abeta42 and tau or phosphorylated tau and prediction of progressive mild cognitive impairment . Neurology . 2005 ; 64 : (7) 1294 - 1297
- 138 . Herukka SK , Helisalmi S , Hallikainen M , Tervo S , Soininen H , Pirttila T . CSF Abeta42, Tau and phosphorylated Tau, APOE epsilon4 allele and MCI type in progressive MCI . Neurobiology of aging . 2007 ; 28 : (4) 507 - 514
- 139 . Brys M , Pirraglia E , Rich K , Rolstad S , Mosconi L , Switalski R , Glodzik-Sobanska L , De Santi S , Zinkowski R , Mehta P . Prediction and longitudinal study of CSF biomarkers in mild cognitive impairment . Neurobiology of aging . 2007 ;
- 140 . DeCarli C . Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment . Lancet neurology . 2003 ; 2 : (1) 15 - 21
- 141 . Ringman JM , Younkin SG , Pratico D , Seltzer W , Cole GM , Geschwind DH , Rodriguez-Agudelo Y , Schaffer B , Fein J , Sokolow S . Biochemical markers in persons with preclinical familial Alzheimer disease . Neurology . 2008 ;
- 142 . Martinez-Yelamos A , Saiz A , Bas J , Hernandez JJ , Graus F , Arbizu T . Tau protein in cerebrospinal fluid: a possible marker of poor outcome in patients with early relapsing-remitting multiple sclerosis . Neuroscience letters . 2004 ; 363 : (1) 14 - 17
- 143 . Jin K , Takeda A , Shiga Y , Sato S , Ohnuma A , Nomura H , Arai H , Kusunoki S , Ikeda M , Itoyama Y . CSF tau protein: a new prognostic marker for Guillain-Barre syndrome . Neurology . 2006 ; 67 : (8) 1470 - 1472
- 144 . Ost M , Nylen K , Csajbok L , Ohrfelt AO , Tullberg M , Wikkelso C , Nellgard P , Rosengren L , Blennow K , Nellgard B . Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury . Neurology . 2006 ; 67 : (9) 1600 - 1604
- 145 . Zetterberg H , Pedersen M , Lind K , Svensson M , Rolstad S , Eckerstrom C , Syversen S , Mattsson UB , Ysander C , Mattsson N . Intra-individual stability of CSF biomarkers for Alzheimer's disease over two years . J Alzheimers Dis . 2007 ; 12 : (3) 255 - 260
- 146 . Blennow K , Hampel H . CSF markers for incipient Alzheimer's disease . Lancet neurology . 2003 ; 2 : (10) 605 - 613
- 147 . Hampel H , Mitchell A , Blennow K , Frank RA , Brettschneider S , Weller L , Moller HJ . Core biological marker candidates of Alzheimer's disease - perspectives for diagnosis, prediction of outcome and reflection of biological activity . J Neural Transm . 2004 ; 111 : (3) 247 - 272

- 148 . Wiltfang J , Lewczuk P , Riederer P , Grunblatt E , Hock C , Scheltens P , Hampel H , Vanderstichele H , Iqbal K , Galasko D . Consensus paper of the WFSBP Task Force on Biological Markers of Dementia: the role of CSF and blood analysis in the early and differential diagnosis of dementia . World J Biol Psychiatry . 2005 ; 6 : (2) 69 - 84
- 149 . Engelborghs S , De Vreese K , Van de Casteele T , Vanderstichele H , Van Everbroeck B , Cras P , Martin JJ , Vanmechelen E , De Deyn PP . Diagnostic performance of a CSF-biomarker panel in autopsy-confirmed dementia . Neurobiology of aging . 2008 ; 29 : (8) 1143 - 1159
- 150 . Bouwman FH , van der Flier WM , Schoonenboom NS , van Elk EJ , Kok A , Rijmen F , Blankenstein MA , Scheltens P . Longitudinal changes of CSF biomarkers in memory clinic patients . Neurology . 2007 ; 69 : (10) 1006 - 1011
- 151 . Bahl JM , Heegaard NH , Falkenhorst G , Laursen H , Hogenhaven H , Molbak K , Jespersgaard C , Hougs L , Waldemar G , Johannsen P . The diagnostic efficiency of biomarkers in sporadic Creutzfeldt-Jakob disease compared to Alzheimer's disease . Neurobiology of aging . 2008 ;
- 152 . Van Everbroeck B , Boons J , Cras P . Cerebrospinal fluid biomarkers in Creutzfeldt-Jakob disease . Clinical neurology and neurosurgery . 2005 ; 107 : (5) 355 - 360
- 153 . Atkinson AJ . Biomarkers and surrogate endpoints: preferred definitions and conceptual framework* . Clin Pharmacol Ther . 2001 ; 69 : 89 - 95
- 154 . Rouzier R , Rajan R , Wagner P , Hess KR , Gold DL , Stec J , Ayers M , Ross JS , Zhang P , Buchholz TA . Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer . Proceedings of the National Academy of Sciences of the United States of America . 2005 ; 102 : (23) 8315 - 8320
- 155 . Zhang B , Higuchi M , Yoshiyama Y , Ishihara T , Forman MS , Martinez D , Joyce S , Trojanowski JQ , Lee VM . Retarded axonal transport of R406W mutant tau in transgenic mice with a neurodegenerative tauopathy . J Neurosci . 2004 ; 24 : (19) 4657 - 4667
- 156 . Zemlan FP , Mulchahey JJ , Gudelsky GA . Quantification and localization of kainic acid-induced neurotoxicity employing a new biomarker of cell death: cleaved microtubule-associated protein-tau (C-tau) . Neuroscience . 2003 ; 121 : (2) 399 - 409
- 157 . Portelius E , Hansson SF , Tran AJ , Zetterberg H , Grognet P , Vanmechelen E , Hoglund K , Brinkmalm G , Westman-Brinkmalm A , Nordhoff E . Characterization of tau in cerebrospinal fluid using mass spectrometry . Journal of proteome research . 2008 ; 7 : (5) 2114 - 2120
- 158 . Vestergaard M , Kerman K , Kim DK , Ha MH , Tamiya E . Detection of Alzheimer's tau protein using localised surface plasmon resonance-based immunochip . Talanta . 2008 ; 74 : (4) 1038 - 1042
- 159 . States DJ , Omenn GS , Blackwell TW , Fermin D , Eng J , Speicher DW , Hanash SM . Challenges in deriving high-confidence protein identifications from data gathered by a HUPO plasma proteome collaborative study . Nature biotechnology . 2006 ; 24 : (3) 333 - 338
- 160 . Bitsch A , Horn C , Kemmling Y , Seipelt M , Hellenbrand U , Stiefel M , Ciesielczyk B , Cepek L , Bahn E , Ratzka P . Serum tau protein level as a marker of axonal damage in acute ischemic stroke . European neurology . 2002 ; 47 : (1) 45 - 51
- 161 . Wunderlich MT , Lins H , Skalej M , Wallesch CW , Goertler M . Neuron-specific enolase and tau protein as neurobiochemical markers of neuronal damage are related to early clinical course and long-term outcome in acute ischemic stroke . Clinical neurology and neurosurgery . 2006 ; 108 : (6) 558 - 563
- 162 . Otto M , Wiltfang J , Cepek L , Neumann M , Mollenhauer B , Steinacker P , Ciesielczyk B , Schulz-Schaeffer W , Kretzschmar HA , Poser S . Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease . Neurology . 2002 ; 58 : (2) 192 - 197
- 163 . Shaw GJ , Jauch EC , Zemlan FP . Serum cleaved tau protein levels and clinical outcome in adult patients with closed head injury . Annals of emergency medicine . 2002 ; 39 : (3) 254 - 257
- 164 . Zemlan FP , Jauch EC , Mulchahey JJ , Gabbita SP , Rosenberg WS , Speciale SG , Zuccarello M . C-tau biomarker of neuronal damage in severe brain injured patients: association with elevated intracranial pressure and clinical outcome . Brain research . 2002 ; 947 : (1) 131 - 139
- 165 . Chatfield DA , Zemlan FP , Day DJ , Menon DK . Discordant temporal patterns of S100beta and cleaved tau protein elevation after head injury: a pilot study . British journal of neurosurgery . 2002 ; 16 : (5) 471 - 476
- 166 . Hu YY , He SS , Wang X , Duan QH , Grundke-Iqbal I , Iqbal K , Wang J . Levels of nonphosphorylated and phosphorylated tau in cerebrospinal fluid of Alzheimer's disease patients : an ultrasensitive bienzyme-substrate-recycle enzyme-linked immunosorbent assay . The American journal of pathology . 2002 ; 160 : (4) 1269 - 1278
- 167 . Kanai M , Matsubara E , Isoe K , Urakami K , Nakashima K , Arai H , Sasaki H , Abe K , Iwatsubo T , Kosaka T . Longitudinal study of cerebrospinal fluid levels of tau, A beta1-40, and A beta1-42(43) in Alzheimer's disease: a study in Japan . Annals of neurology . 1998 ; 44 : (1) 17 - 26
- 168 . Andreasen N , Minthon L , Clarberg A , Davidsson P , Gottfries J , Vanmechelen E , Vanderstichele H , Winblad B , Blennow K . Sensitivity, specificity, and stability of CSF-tau in AD in a community-based patient sample . Neurology . 1999 ; 53 : (7) 1488 - 1494
- 169 . Sunderland T , Wolozin B , Galasko D , Levy J , Dukoff R , Bahro M , Lasser R , Motter R , Lehtimaki T , Seubert P . Longitudinal stability of CSF tau levels in Alzheimer patients . Biological psychiatry . 1999 ; 46 : (6) 750 - 755
- 170 . Blennow K , Zetterberg H , Minthon L , Lannfelt L , Strid S , Annas P , Basun H , Andreasen N . Longitudinal stability of CSF biomarkers in Alzheimer's disease . Neuroscience letters . 2007 ; 419 : (1) 18 - 22
- 171 . Sanchez-Juan P , Sanchez-Valle R , Green A , Ladogana A , Cuadrado-Corrales N , Mitrova E , Stoeck K , Sklaviadis T , Kulczycki J , Hess K . Influence of timing on CSF tests value for Creutzfeldt-Jakob disease diagnosis . Journal of neurology . 2007 ; 254 : (7) 901 - 906
- 172 . Hesse C , Rosengren L , Andreasen N , Davidsson P , Vanderstichele H , Vanmechelen E , Blennow K . Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke . Neuroscience letters . 2001 ; 297 : (3) 187 - 190
- 173 . Van Gool SW , Van Kerschaver E , Brock P , Pottel H , Hulstaert F , Vanmechelen E , Uyttebroeck A , Van De Voorde A , Vanderstichele H . Disease- and treatment-related elevation of the neurodegenerative marker tau in children with hematological malignancies . Leukemia . 2000 ; 14 : (12) 2076 - 2084
- 174 . Parnetti L , Amici S , Lanari A , Romani C , Antognelli C , Andreasen N , Minthon L , Davidsson P , Pottel H , Blennow K . Cerebrospinal fluid levels of biomarkers and activity of acetylcholinesterase (AChE) and butyrylcholinesterase in AD patients before and after treatment with different AChE inhibitors . Neurol Sci . 2002 ; 23 : (Suppl 2) S95 - 96
- 175 . Degerman Gunnarsson M , Kilander L , Basun H , Lannfelt L . Reduction of phosphorylated tau during memantine treatment of Alzheimer's disease . Dementia and geriatric cognitive disorders . 2007 ; 24 : (4) 247 - 252
- 176 . Gilman S , Koller M , Black RS , Jenkins L , Griffith SG , Fox NC , Eisner L , Kirby L , Rovira MB , Forette F . Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial . Neurology . 2005 ; 64 : (9) 1553 - 1562
- 177 . Riekse RG , Li G , Petrie EC , Leverenz JB , Vavrek D , Vuletic S , Albers JJ , Montine TJ , Lee VM , Lee M . Effect of statins on Alzheimer's disease biomarkers in cerebrospinal fluid . J Alzheimers Dis . 2006 ; 10 : (4) 399 - 406
- 178 . Borroni B , Yancopoulou D , Tsutsui M , Padovani A , Sawcer SJ , Hodges JR , Spillantini MG . Association between tau H2 haplotype and age at onset in frontotemporal dementia . Archives of neurology . 2005 ; 62 : (9) 1419 - 1422
- 179 . Zhang B , Maiti A , Shively S , Lakhani F , McDonald-Jones G , Bruce J , Lee EB , Xie SX , Joyce S , Li C . Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model . Proceedings of the National Academy of Sciences of the United States of America . 2005 ; 102 : (1) 227 - 231

Figure 1

Stages in the neuropathology of Alzheimer's disease. Implication for the use of Tau as biomarker during the course of the disease

AD lesions, namely neurofibrillary degeneration, follow a stereotyped, sequential, hierarchical pathway. The progression is categorized into 10 stages according to the brain regions affected: transentorhinal cortex (S1), entorhinal (S2), hippocampus (S3), anterior temporal cortex (S4), inferior temporal cortex (S5), medium temporal cortex (S6), polymodal association areas (prefrontal, parietal inferior, temporal superior) (S7), unimodal areas (S8), primary motor (S9a) or sensory (S9b, S9c) areas, and all neocortical areas (S10). Up to stage 6, the disease can be asymptomatic or paucisymptomatic (MCI): the CSF levels of pTau are however already altered and are useful for predictive diagnosis. In the more advanced stages when clinical criteria of AD are fulfilled CSF-tTau and CSF-pTau are altered and stable during disease course. Only the post-mortem analysis of brain with the presence of the two characteristic lesions of AD (amyloid plaques and NFD) allows to obtain the definite diagnosis of AD. The electrophoretic analysis of PHF-Tau aggregates is a useful tool for differential diagnosis of AD in brain tissue.

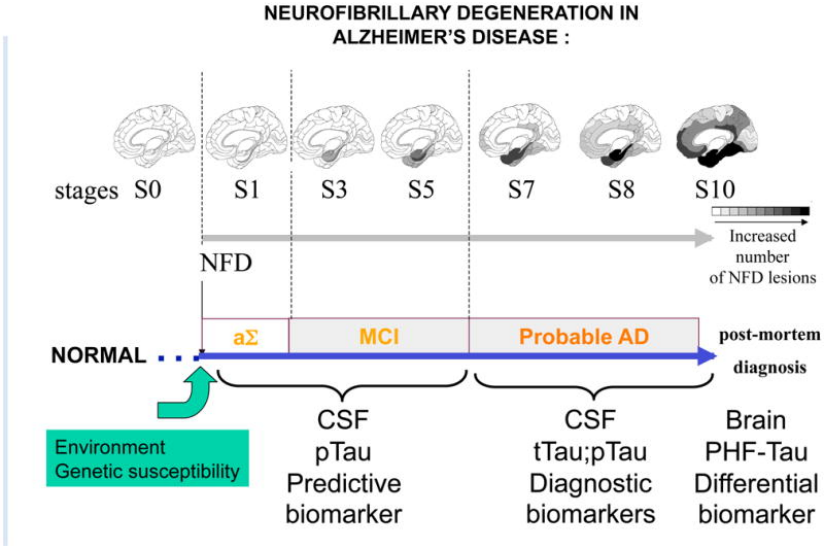

Figure 2

Microtubule-associated Tau gene, RNAs and human brain isoforms

(A) Genomic architecture of the 17q21.31 region encompassing MAPT gene and flanked by low copy repeats (rectangles) that are susceptible to chromosomal rearrangements such as deletions, duplications or inversion. Dotted lines illustrate the breakpoints responsible for the inversion of a ~900kb segment resulting in the H1/H2 polymorphism. (B) Tau gene MAPT spans more than 130 kb and is composed of 16 exons. The most studied polymorphism associated to the H1/H2 haplotype are 8 SNP (⬥), 1 (TG)n microsatellite ({}) and a 238pb insertion/deletion (◇).H1 haplotype is associated with PSP and CBD. The three most frequent mutations responsible of FTDP-17 are in red. (C) Several mRNAs are generated by alternative splicing of exons 2, 3, 4A and 10. (D) In the human brain, six major Tau isoforms are generated from the alterative splicing of exons 2, 3 and 10. Exon 3 is always included with exon 2. The exon 10 encodes an additional microtubule-binding motif numbered R1 to R4. Half of Tau proteins contain three microtubule-binding motifs and the other half has four microtubule-binding motifs.

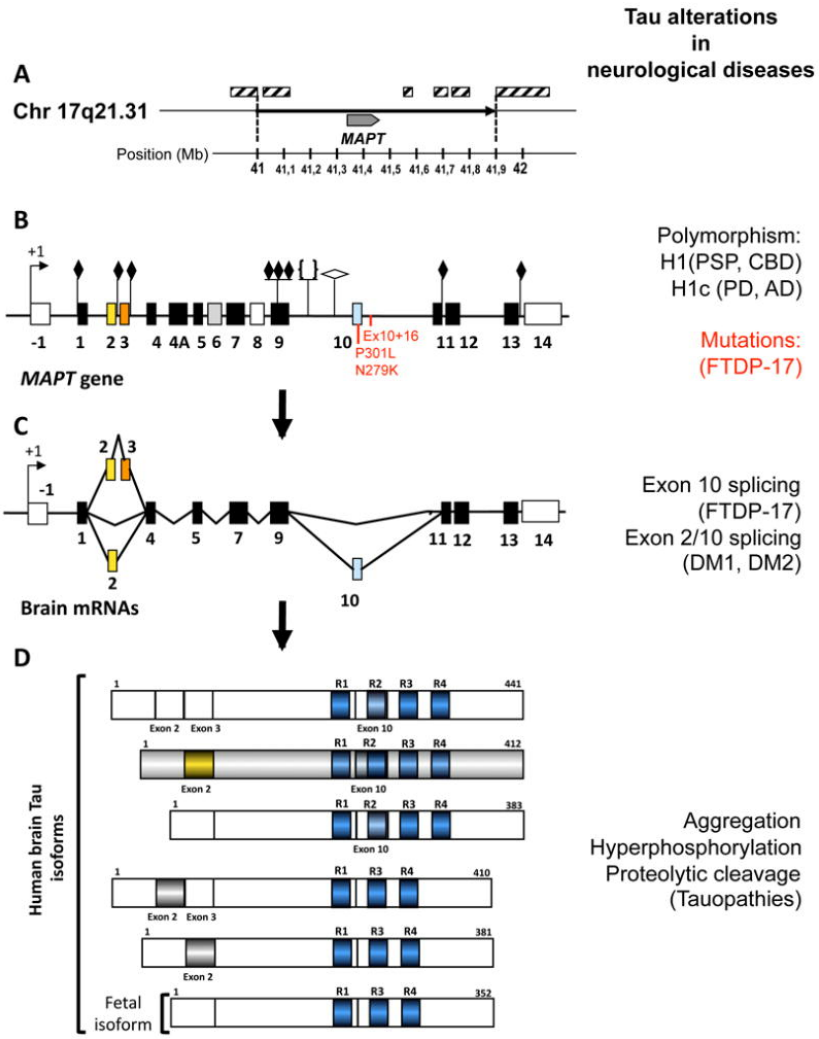

Figure 3

Semi-quantitative analysis of Tau exon 2 in control and DM1 brains

Reduced Tau exon 2 inclusion in DM1 brains. Tau exons 2 and 3 are alternatively spliced. Splicing of these two exons generated 3 Tau transcripts named 2+3+, 2+3- and 2-3-. The proportion of Tau transcripts including exon 2 and 3 was analysed by RT-PCR in three brain area (hippocampus, temporal and frontal cortex) in two control and in one DM1 patients. (B) Histogram of the percentage of transcripts including exon 2. The bands were quantified and the results expressed as the percentage of transcripts including exon 2. The mean and standard deviation were calculated from three individual experiments. Histograms are representative of the mean +/- standard deviation of three independent experiment.

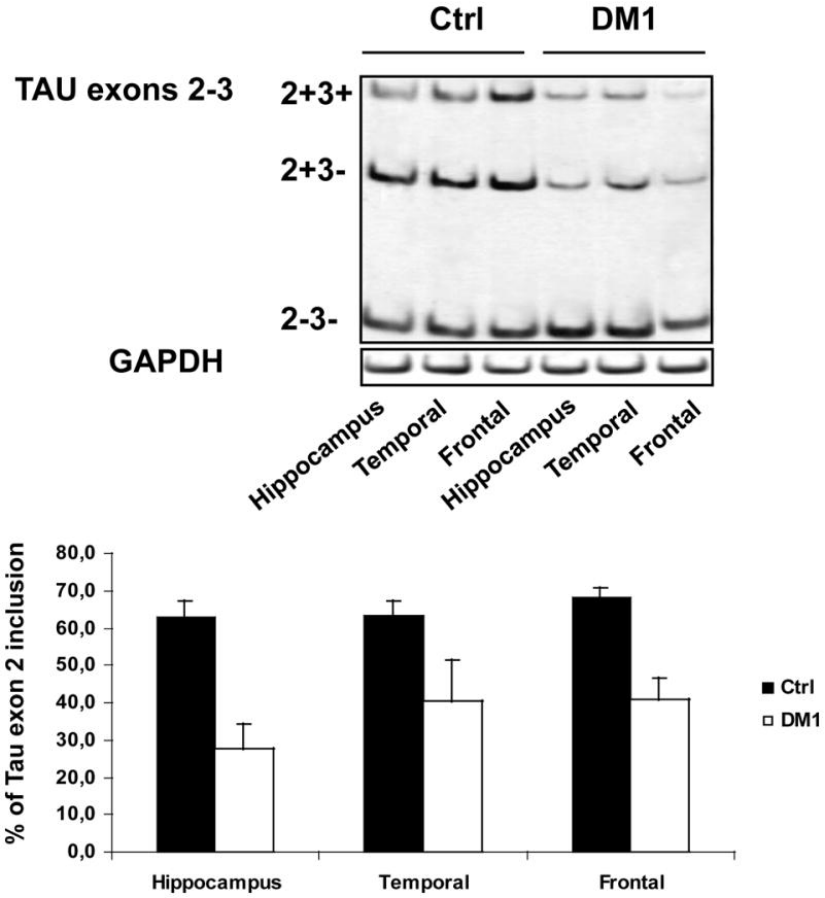

Figure 4

The “Bar code” of neurodegenerative diseases

Aggregated Tau proteins from the brain tissue of patients suffering from differents neurodegenerative disorders were resolved by 1D gels. Four main patterns of Tau bands were observed and the isoform content was determined using Tau exon-specific antibodies or two-dimensional gel electrophoresis. A classification is proposed according to Tau isoforms composing each of four biochemical patterns. A. Class I, which encompasses the largest number of degenerative diseases with Tau aggregation, is characterized by the aggregation of six Tau isoforms. This class is characterized by the occurrence on the brain samples of the hallmarks of Alzheimer's disease i.e 1) intraneuronal somatic neurofibrillary tangles (NFT) as shown on the left panel by an antibody against pSer396-404 of Tau (AD2, arrows), 2) neuropile threads (NT), corresponding to neuritic processes filled of aggregated Tau (asterisks) and 3) neuritic plaques (NP). Antibodies raised against the sequences coded either by exon 2 (Tau E2, upper right panel) or by exon 10 (Tau E10, lower right) stain similarly NFTs(arrows), NTs (asterisks) and NPs (arrowhead) in AD and in other class I tauopathies. B. Only Tau isoforms containing four microtubule-binding domains aggregate in Class II disorders. They are represented here by a PSP case. AD2 anti-pTau labels the so-called astrocytic tufts, corresponding to intra-cytoplasmic Tau aggregation in astrocytes (arrow, left panel). Tau E2 and Tau E10 show glial Tau pathology as well, astrocytic (upper right, arrow) or oligodendrial (arrowhead, lower right). The “globoïd” neurobibrillary tangles are seen here in the substantia nigra (upper right, asterisk) and are stained by both Tau E2 and Tau E10. C. Tau isoforms with three microtubule-binding domains are found in Pick's disease and few FTDP-17, corresponding to Class III diseases. Pick bodies are stained by anti-pTau and Tau E2 (arrows showing spherical 10µm bodies) but not by Tau E10 (in the lower right panel, a Pick body is readily seen but unstained, asterisk) D. At last, Class IV is characterized by the aggregation of Tau isoforms lacking sequences encoded by exons 2 and 3. Uptoday, the only known class IV diseases DM1 and DM2. On the left panel aggregates of hyperphosphorylated Tau are labelled with AD2. On the right panel, only unphosphorylated, non aggregated normal Tau are stained by anti-Tau E2 which in turn labels no aggregate.

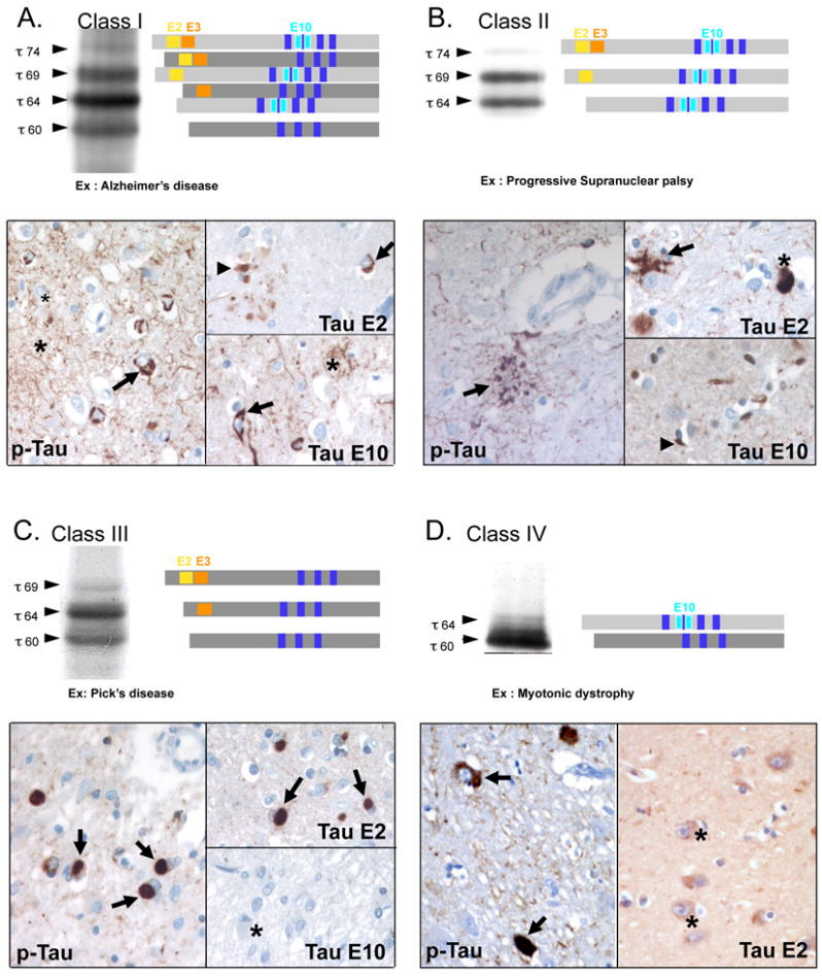

Figure 5

Characterization of Tau in cerebrospinal fluid

Sequence of the largest form of brain-derived Tau, containing all alternatively spliced exons, is shown. Boxes in colour are the alternatively spliced exons and the microtubule-binding repeats: yellow, exon2; green exon 3, light blue exon 10 and the boxes in blue are the four tubulin-binding repeat domains Boxes indicate the epitopes of some antibodies commonly used i) in immuno-assays to quantify Tau in human CSF: for total Tau HT7, AT120 and BT2 are used, HT7-AT270, Tau-1-PHF-1 and Tau-1/CP27 are used in phospho-specific assays. [116 , 166] ii) in brain tissue analysis : AD2, AT100 or 12E8 for Tau phospho-epitopes. Letters in red indicate the 8 sequences identified using an optimized immunoprecipitation (BT2) mass spectrometry analysis of 16 peptides [157].

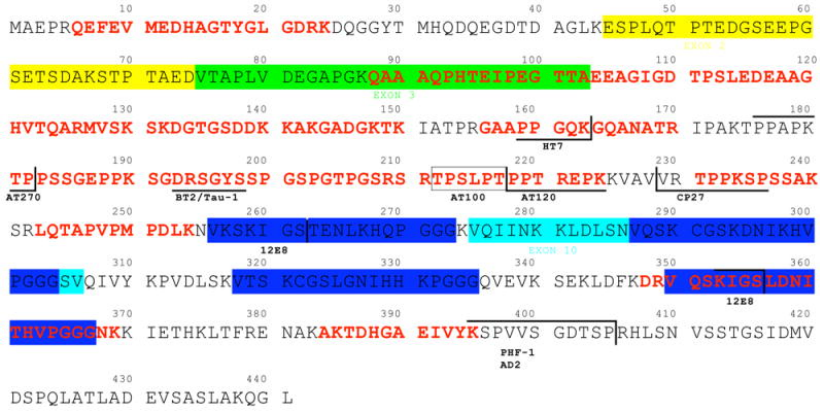

Table 1

Follow-up studies of Tau and pTau levels during chronic and acute neurodegenerative disorders.

Study	Diagnostic group	Follow-up	tTau baseline (pg/ml)	tTau follow-up	Ptau baseline (pg/ml)	Ptau follow-up
Stable Tau/pTau levels in CSF of AD and CJD patients						
[167]	9 AD	18.6 mo (2 – 43)	847 ± 253	878 ± 452	NA	
[168]	192 AD	1 yr	21% CV		NA	
[169]	29 AD	2 yrs	548 ± 355	577 ± 275	NA	
[135]	8 MCI	1 yr			530 ± 450*	560 ± 480*
[123]	20 AD	3-8 yrs	568 ± 365	570 ± 435	NA	
[170]	53 AD	6 mo	425 ± 30	431 ± 30 (6.1% CV)	93 ± 6	94 ± 6 (4.4% CV)
[150]	50 AD	21 ± 8 mo	731 ± 363	791 ± 419	84 ± 34	85 ± 35
[139]	22 MCI-AD	2.1 ± 0.5	750 ± 553	667 ± 327	49 ± 31	52 ± 29
[145]	12 MCI-AD	2 yrs	700 (240 -1700)	710 (270 – 1300)	86 (47-150)	86 (48-150)
[171]	66 CJD	6 wks	tTau Not significant		NA	
Transient increase in CSF-tTau/pTau levels in conditions with neuronal/axonal loss						
[172]	Brain trauma, n=9		770 ± 160 (wk 3)	250 ± 40 (3-5 mo)	37 ± 8 (wk 3)	29 ± 3 (3-5 mo)
[173]	Neurotoxicity, n=6		535-776 on day 8	<300 after 200 days	NA	
[99]	Alcoholic dementia		CSF-tau/P-tau significantly elevated in acute Wernicke's encelopathy (WE, n=10) but not in chronic WE and after longterm follow-up			

Table 2

Tau as a potential surrogate biomarker: from mRNA to protein

As a protein Tau/pTau in CSF has been monitored in AD patients treated with AD drugs, while the effect of SNP-gene expression and mRNA have only been observed in clinical conditions and drug treatments in other conditions or in a transgenic animal.

Tau	Drug	Effect	Reference
Protein	AChE-inhibitors (exelon, rivastigmine, galantamine)	No effect on CSF-tTau/Ptau levels	[174]
	Memantine	Decreased (=normalization) CSF-pTau levels (n=11)	[175]
	AN1792 (Aβ vaccine)	CSF-tau levels decreased in antibody responders (n=11) vs placebo subjects (n=10, p<0.001)	[176]
	Statins	Simvastatin-treatment, but not pravastatin, results in reduced CSF-pTau levels in all subjects (n=10)	[177]
SNP-gene expression		Tau haplotype affects CSF-tTau levels in FTD	[178]
		Tau haplotype is associated with increased 4-repeat tau gene expression in brain	[34]
		Minor SNP alleles are associated with increased CSF-tTau/pTau levels in the presence of amyloidbeta deposition	[126]
mRNA	Paclitaxel (taxanes) for treatment of breast cancer	Tau mRNA down-regulation is sensitive to drug	[154]
	Paclitaxel in PrP T44 Tg mice	Ameliorate motor impairment	[179]